Ten2Twenty-Ghana: Study Design and Methods for an Innovative Randomized Controlled Trial with Multiple-Micronutrient–Fortified Biscuits among Adolescent Girls in Northeastern Ghana

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ABSTRACT

Investing in adolescent girls' nutrition is vital for health and for breaking the intergenerational cycle of malnutrition and deprivation, but limited knowledge on the type, timing, and efficacy of interventions delays progress. We describe the design of a 26-wk randomized placebo-controlled trial with multiple-micronutrient–fortified biscuits (MMBs) among adolescent girls in northeastern Ghana. Apparently healthy, premenarche ($n = 312$) and postmenarche ($n = 309$) girls (10–17 y) were randomly assigned to receive the following for 5 d/wk: *1*) MMBs (fortified with 11 vitamins and 7 minerals) or *2*) unfortified biscuits. Data included plasma micronutrient status, anthropometry, body composition, cognitive function, psychosocial health, fertility, dietary intake, and sociodemographic and socioeconomic covariates, complemented with in-depth interviews ($n = 30$) and 4 focus group discussions. We hypothesized an increase in plasma ferritin and retinol-binding protein with a resultant increase in hemoglobin, cognition, vertical height, and psychosocial health. Our study seeks to investigate the efficacy and optimal timing of a multiple-micronutrient food intervention program for adolescent girls. The RCT was registered prospectively with the Netherlands Clinical Trials Register (NL7487). *Curr Dev Nutr* 2021;5:nzaa184.

Keywords: Ghana, adolescent girls, malnutrition, menarche, multiple-micronutrients, fortified biscuits, body composition, nutrient gaps

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Supplemental material is available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/cdn/.

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Abbreviations used: AAM, age at menarche; AE, adverse event; AGP, α-glycoprotein; CRP, C-reactive protein; DDS, Dietary Diversity Score; FBDG, food-based dietary guideline; FFQ, food-frequency questionnaire; GES, Ghana Education Service; GHS, Ghana Health Service; GIFTS, Girls' Iron Folate Tablet Supplementation; GSS, Ghana Statistical Service; Hb, hemoglobin; HRQoL, health-related quality of life; ID, iron deficiency; IDA, iron deficiency anemia; KAP, knowledge, attitudes, and practices; LMIC, low- and middle-income country; LMM, linear mixed model; MMB, multiple-micronutrient–fortified biscuit; MMF, multiple-micronutrient–fortified food; NIH-TCB, NIH toolbox cognition battery; RBP, retinol-binding protein; RCT, randomized controlled trial; RE, retinol equivalents; SAE, severe adverse event; SF, plasma serum ferritin; SSA, sub-Saharan Africa; TfR, plasma soluble transferrin receptor; TTH, Tamale Teaching Hospital; UB, unfortified biscuit; UDS, University for Development Studies; VAD, vitamin A deficiency; WUR, Wageningen University and Research; 24hR, quantitative 24-h dietary recall.

Introduction

Adolescents make up the most significant proportion (23%) of the population in sub-Saharan Africa (SSA), and this subregion is projected to have more adolescents than in any other region by 2050 (1). According to the Ghana Statistical Service (GSS), approximately one-quarter of the Ghanaian population are adolescent (2) and a little over one-fifth of the female Ghanaian population are adolescents (2). The WHO defines adolescence as the life stage of ∼10–19 y (3).

In addition to the first 1000 d of life, adolescence offers an additional (and last) window of opportunity to improve nutrition and health (4–6). Adolescence is the only other time in life when the velocity of growth increases, as ∼45% of total skeletal mass and 15–25% of adult height are gained during adolescence (7, 8). As a result of the growth spurt, adolescents' energy and protein requirements are highest compared with any other age group (8, 9). Similarly, micronutrient requirements, mainly iron, calcium, zinc, and vitamin D, increase during adolescence (10), leaving adolescents vulnerable to micronutrient deficiencies (11, 12), especially in resource-poor areas. For girls, age at menarche (AAM) is an essential landmark, considering that most height is attained before menarche (13, 14). Additional height is obtained at a lower velocity over 4.7 y after menarche (13, 14). Likewise, pelvic bone growth, critical for preventing birth and pregnancy complications, is gained just before, and for 4.7 years after, menarche (13, 14).

Menstruation may probably induce a higher iron intake through the homeostatic mechanism of upregulated iron during deficiency (15, 16), making postmenarche girls benefiting most from a micronutrient intervention. However, menstruation is proinflammatory (17), a condition that negatively affects micronutrient status, notably iron and vitamin A status (18, 19), implying that postmenarche girls may benefit less from an intervention. These opposite processes highlight the uncertainty of a nutrition intervention's best timing, either pre- or postmenarche. A higher BMI and fat mass are also associated with a lower AAM (20); but little is known about the association between nutrient status and AAM.

Generally, girls in Ghana are disadvantaged in intrahousehold food distribution and resource allocation (21), and are at risk of sexual violence (22). Girls are also less educated than boys (23, 24) and are more likely to drop out of secondary school than boys (23); for instance, only 78.7% of girls complete primary education compared with 92.4% of boys in the Northern Region of Ghana (25). Last, approximately one-third of Ghanaian adolescent girls are married by age 18 (2), and 14% of adolescents aged 15–19 in Ghana have begun childbearing (26). Furthermore, malnutrition is prevalent among adolescent girls in Ghana. The 2014 Ghana Demographic and Health Survey (26) indicates that 14.4% of 15- to 19-y-old female adolescents are too thin, and 8.7% are overweight. Also, there is increasing evidence of inadequate micronutrient status among girls (27, 28), and particularly iron deficiency anemia (IDA) (17), a result of the increased iron needs during periods of rapid growth and blood loss during menstruation after menarche (17, 29). In a recent analysis, ∼64.6% of rural adolescent girls were anemic in the northern savannah agro-ecological zone of Ghana (30). Despite the increasing evidence of inadequate micronutrient status among adolescent girls, nutrition intervention programs in Ghana, like in other developing contexts, have commonly focused on infants, young children, and pregnant and lactating women, and generally neglected adolescents.

The diet of most adolescents in low- and middle-income countries (LMICs) mostly consists of low consumption of fruits and vegetables and high intake of starch, sweetened foods and beverages, and snacks in the form of fast foods (31). Nonetheless, little is known about nutrient gaps in adolescent girls' diets, as information on dietary practices and nutrient intakes is rarely collected for adolescents in LMICs (32), and Ghana is no exception.

These deprivations predispose adolescent girls in Ghana to malnutrition, undermining their development into adulthood. Malnutrition perpetuates a vicious cycle of malnutrition, poor health, and poverty and as well affects the health and well-being of future generations because of the intergenerational transfer of (dis)advantages regarding health and development and inequities therein. In particular, attention to adolescent girls' nutrition is vital to improving girls' health status and that of their (future) offspring, thereby breaking intergenerational cycles of malnutrition and deprivation (4, 6, 33). Indeed, adolescence is seen as the second (and last) window of opportunity to improve nutritional and developmental outcomes because of the pubertal growth spurt and formative processes that occur during this life phase (5, 34).

For settings with a high burden of anemia and IDA, the WHO recommends intermittent iron and folic acid supplementation for menstruating women and adolescents (35). Nonetheless, poor compliance often limits the effectiveness of micronutrient supplementation programs (36, 37). Food fortification without changes in food's organoleptic properties is potentially useful for improving adolescent girls' nutrition and health, but evidence from LMICs is scarce. However, there is existing evidence from developed countries that the consumption of multiple-micronutrient fortified foods (MMFs) improves micronutrient status and reduces nutritional deficiencies among adolescent girls (38–42). A few emerging studies from LMICs, mainly in South-East Asia, also affirm this association (43–45). To our knowledge, no study from SSA has explored the effect of MMFs on the micronutrient status and health of adolescent girls. Biscuits may be a convenient food vehicle for fortification as they are handy and easy to manage. More so, biscuits are more likely to be accepted by the adolescent population who often prefer snacks (46).

Undernutrition during adolescence has adverse consequences for cognition, psychosocial health, and the socioeconomic life trajectories of adolescents. Like infants and young children, inadequate intake also increases the risk of infections, contributing to a vicious cycle of undernutrition, infection, and poor developmental outcomes (47, 48). According to Mesías et al. (17), iron deficiency (ID), besides provoking significant physiological consequences, also adversely affects adolescents' cognitive ability and motor and mental development. For instance, Robinson and colleagues (49), in a follow-up study, illustrated that anemia, ID, and vitamin B-12 deficiency are associated with adolescent boys' behavioral problems in Bogota. Iron provides oxygen to the brain via erythropoiesis, and for myelination of the frontal lobes, which notably occurs during adolescence (50, 51). Iron is also a cofactor for enzymes involved in neurotransmitter synthesis and plays a role in neurotransmitter metabolism (51). Also, iodine is critical for neuronal cell maturation, and myelination and a deficiency can impair adolescents' cognitive function (52).

Additionally, vitamin A influences thyroid metabolism alongside iodine, and vitamin A is also required for erythropoiesis (53). A plausible mechanism through which micronutrient problems may result in cognitive and behavioral problems during adolescence involves poor myelination and reduced oligodendrocyte function (54). ID has been shown to alter neurochemical metabolism, such as phosphocreatine, glutamate, N-acetyl aspartate, aspartate, and γ-aminobutyric acid in the hippocampus, which may trigger some cognitive and mental problems.

A few studies have also shown that improved nutrition outcomes during adolescence are positively associated with improved psychosocial health, notably health-related quality of life, self-esteem, self-efficacy (55–57), cognitive skills (58–60), educational performance (59, 61, 62), and family formation (63). However, the evidence has mainly been cross-sectional, making it somewhat impossible to establish causality. The existing studies in SSA have mainly been conducted in Ethiopia (55, 58, 61) and Uganda (62), and none of these studies examined the effect of micronutrient status other than anemia on psychosocial health and cognition.

Throughout the transition to adulthood, girls' nutritional trajectories (e.g., nutritional status, dietary intake) are interwoven with social and economic trajectories, including education, family formation, and labor participation (64–67). These parallel trajectories are often interlinked, and the encounter and accumulation of disadvantages (representing a life-course perspective) within the social, economic, and nutritional trajectories may negatively impact girls' health, well-being, and opportunities (66, 67), with resultant low social and economic status within the household for girls. Understanding context-specific trajectories and the interactions and interrelations is crucial to improving girls' nutritional exposure and outcomes in multiple life domains. Nutrition may influence adolescents' aspirations through the

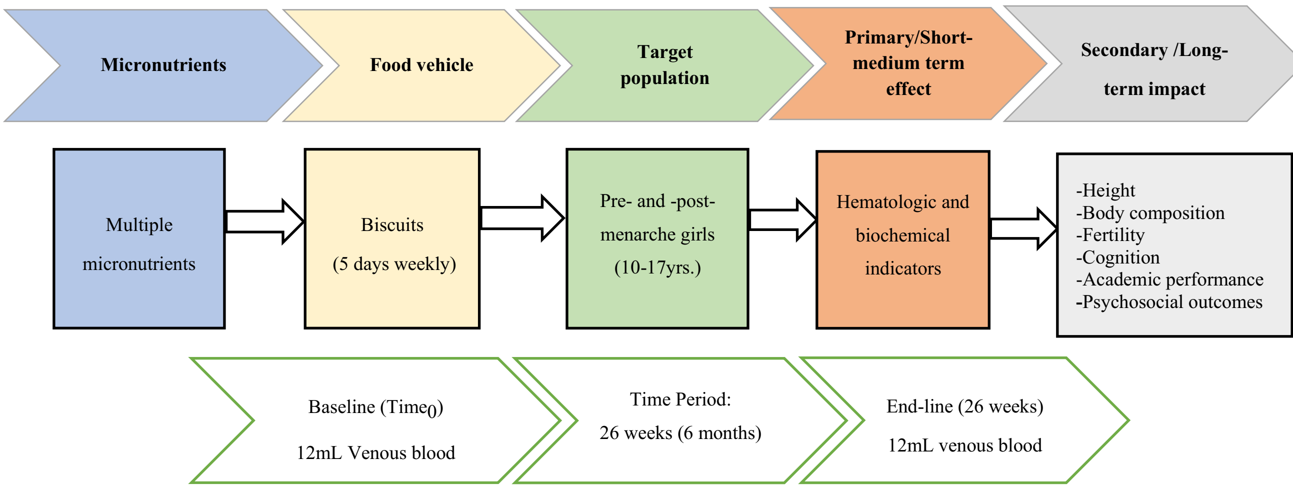

FIGURE 1 Logic framework for the effect of a multiple-micronutrient–fortified biscuit intervention for adolescent girls.

intergenerational transfer of (dis)advantages and cognition and psychosocial health pathways, including mental health (55, 68). For instance, ID leads to fatigue and lethargy and may affect aspirations. Feeling energetic may also increase hopes for the future. However, girls' perspectives on growing up and their future aspirations and the numerous life domains remain unexplored.

According to the WHO (33), the lack of age- and sex-specific data on adolescent girls' and boys' health and nutritional status is the primary reason for the typical lack of policies and programs that can improve adolescents' health and nutritional status. Such data are urgently needed to help build a common ground for the inclusion of MMFs in programs towards improving adolescent girls' health in developing contexts like Ghana.

Furthermore, a mother's nutritional status and her participation in household decision making, a proxy for empowerment, are known determinants of improved nutrition and health outcomes for infants and young children (69–71). However, little is known about the influence of a mother's nutrition and her participation in household final decision making on adolescents' nutrition and health. We aim to contribute to these existing knowledge gaps by designing the innovative research entitled "Ten2Twenty-Ghana" using a mixed-methods approach for data collection. The primary research question is as follows:

1. What is the effect of consuming multiple-micronutrient–fortified biscuits (MMBs) compared with unfortified biscuits (UBs) 5 d weekly for 26 wk on biomarkers of micronutrient status of adolescent girls and how is the intervention effect related to its timing (before or after menarche)?

Secondary research questions include the following:

1. How do the intervention [randomized controlled trial (RCT)] and its timing relate to changes in psychosocial health, cognitive functioning, academic performance, and fertility of the adolescent girls?
2. How do the intervention and its timing relate to changes in the adolescent girls' vertical growth and body composition?

Overall, we hypothesized that a fortified-food intervention program using MMBs would improve micronutrient status in the short to medium term, with changes in the secondary outcomes in the medium to long term (**Figure 1**).

The study also includes 3 additional research questions, including the following:

1. What is the dietary intake and its determinants of adolescent girls in different pubertal stages (before/after menarche)?
2. What affordable, evidence-based, population-specific food-based dietary guidelines (FBDGs) can fulfill or best meet adolescent girls' nutrient requirements in Ghana?
3. How do the mother's nutritional status and her participation in household decision making influence the adolescent girl's nutrition and health?

Methods

Study area

We conducted the study in the Mion District, in Northeastern Ghana. The district is located in the eastern corridor of the Northern Region of Ghana between latitude 90–35° north, 00–30° west, and 00–15° east. The district shares boundaries with the Tamale Metropolis, Savelugu Municipal, and Nanton District to the west; Yendi Municipal to the east; Nanumba North and East Gonja districts to the south; and Gushegu and Karaga districts to the north. The district capital is Sang, the largest community in the district. The district covers a surface area of 2714.1 square km and has a population density of 30.1 persons per square kilometer (72). The area has a typical tropical climate with 2 main seasons: a dry season (November–March), characterized by high temperatures, and a single rainy season (April–October). According to the 2010 Ghana Population and Housing Census (72), Mion District had a population of 81,812, with the majority (91.1%) living in rural locations. In 2010, the average household size in the district was 9.3 persons per household. According to the GSS, ~20% of the district's female population was aged 10–19 y (72). The district's main ethnic groups are Dogombas and Konkombas, and ~61.8% of the district population professes Islam. Over 90% of the people depend on agriculture for their livelihood. In 2010, the district's literacy rate was 28.7%

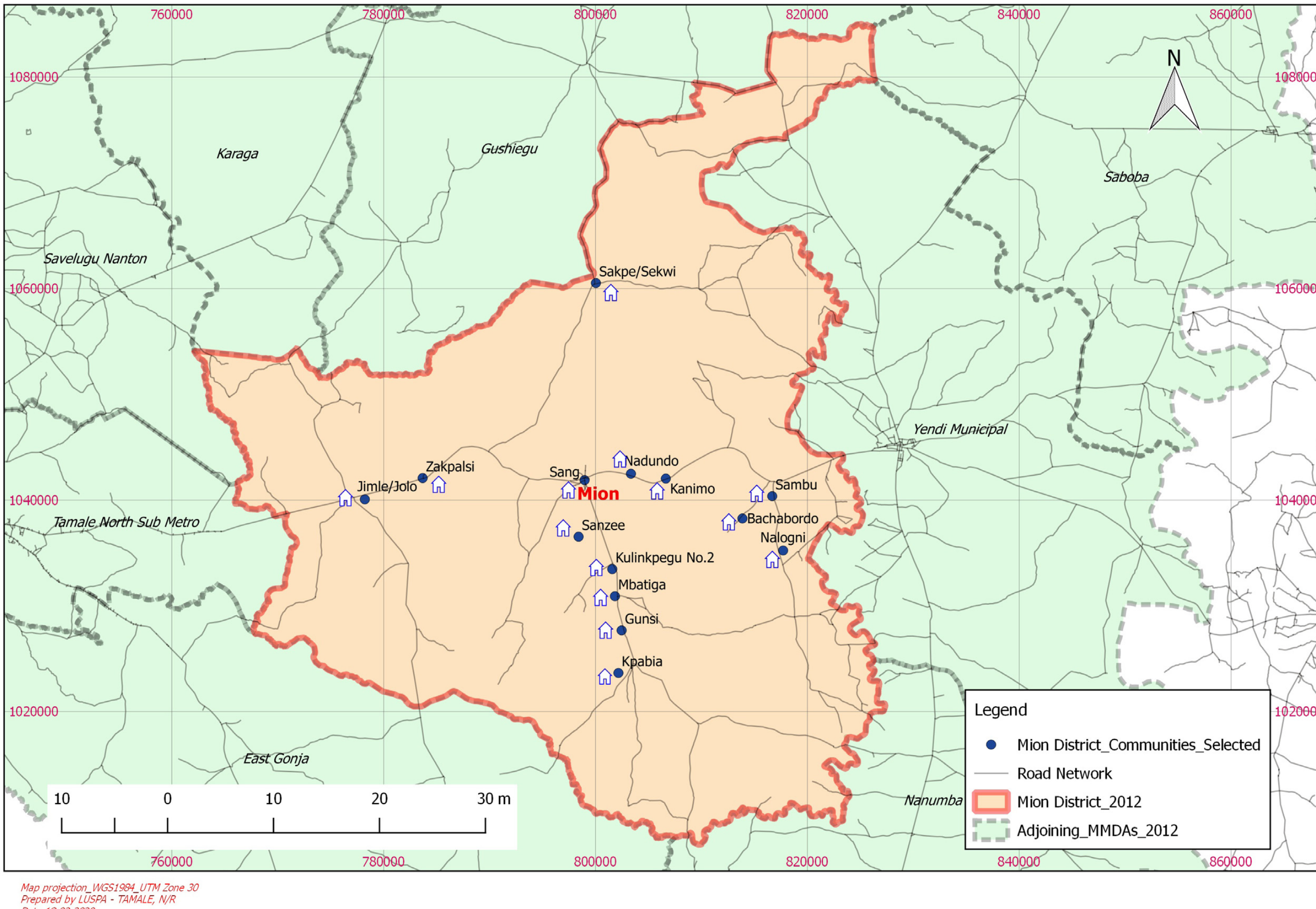

FIGURE 2 Map of Mion District, Ghana, with the communities included in the Ten2Twenty-Ghana study. MMDAs, Metropolitan and Municipal Assemblies.

for both sexes, implying a very high illiteracy rate in the district (72). The study protocol was approved by the Navrongo Health Research Centre Institutional Review Board (NHRCIRB323). The RCT was also registered prospectively with the Netherlands Clinical Trials Register (NL7487).

We purposively selected Mion District as it is relatively new, carved out of Yendi Municipal Assembly in 2012. Hence, data on nutrition and health in the district are scanty. Moreover, the district is mainly rural (~91%), and our secondary analysis (30) suggests a very high prevalence (64.6%) of anemia among adolescent girls in the rural northern savannah agro-ecological zone. Last, the district capital is only ~1 h drive from Tamale, the regional capital and location of the University for Development Studies (UDS), which coordinated the fieldwork. Figure 2 is a map of the district with locations of the selected communities where the study was conducted.

Study design

The study started with an extensive cross-sectional survey ($n = 1057$), 2 mo before a follow-up double-blind, placebo RCT. Herein, we refer to the cross-sectional survey as the "survey," and we describe the methods for the survey and RCT in this article. For ethical reasons, a nontargeted approach that did not distinguish anemic and nonanemic girls was used to include a random subsample ($n = 620$) of girls from the RCT survey. The nontargeted approach was also justified by the high prevalence of anemia (64.6%) among female adolescents in the rural northern savannah agro-ecological zone of Ghana (30). The nontargeted approach was previously used in similar efficacy trials and proved to be effective (43, 73, 74). The girls were randomly assigned to 2 parallel treatment arms receiving nutrition/health education (5 different occasions) with a 5-d weekly MMBs or UBs for 26 wk. Similar studies (40–45) reported significant effects of MMFs on children's and adolescents' micronutrient status when consumed between 5 and 7 d for 3–12 mo with an average duration of 6 mo; this informed our decision to administer the treatment 5 d/wk for 6 mo. Figure 3 shows a schematic overview of the RCT. To estimate mean nutrient intake and the proportion of the population at risk of nutrient inadequacy, a random subsample ($n = 310$) of subjects from the RCT (including both pre-and postmenarche girls) was selected for a quantitative 24-h dietary recall (24hR). Out of the first 24hRs, a random sample of 100 girls was selected for a second 24hR, allowing us to adjust for

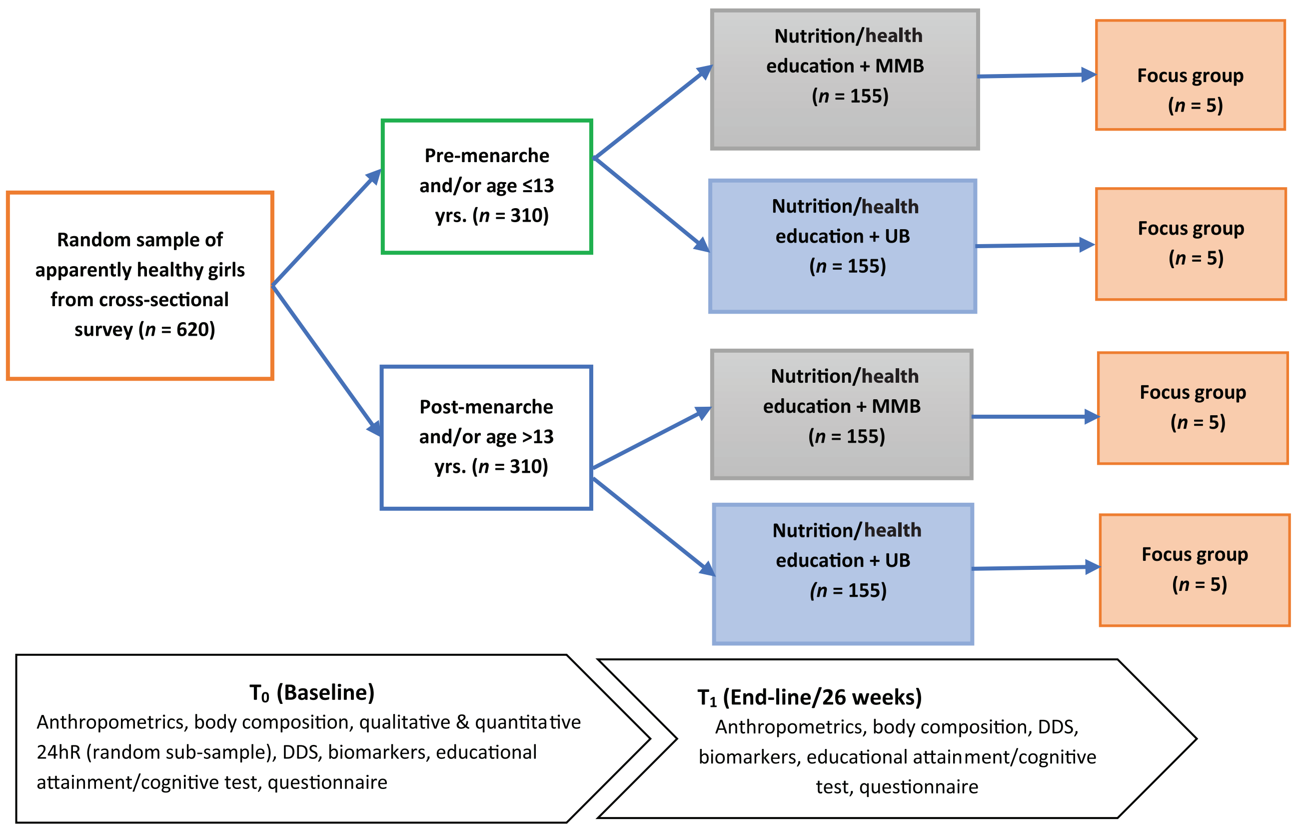

FIGURE 3 Design of a 26-wk, double-blind, randomized placebo-controlled trial among adolescent girls in Ghana. DDS, Dietary Diversity Score; MMB, multiple-micronutrient fortified biscuits; UB, unfortified biscuits; 24hR, quantitative 24-h dietary recall.

the random day-to-day variation in dietary intake. For triangulation purposes, we also conducted 1 focus group discussion in each of the RCT arms at the endline. Likewise, in the extensive cross-sectional survey, we conducted qualitative in-depth interviews ($n = 30$) and 2 focus group discussions. **Supplemental material** indicates the SPIRIT checklist for the study.

Study population

The target population for the study included premenarche and postmenarche adolescent girls. The girls were seemingly healthy, nonpregnant, and nonlactating adolescents aged 10–17 y, residing in the Mion District in the Northern Region of Ghana. To be enrolled into the survey, girls had to meet all of the inclusion criteria, and those who did not meet the exclusion criteria of the RCT were eligible to participate in the RCT (**Table 1**).

Sample size estimation

The survey sample ($n = 1040$) was estimated to detect a minimum mean difference of 0.30 in math and verbal skills z scores between stunted and nonstunted adolescent girls (60) using the RMASS program (http://www.rmass.org/) (75). The RCT sample size calculation was based on 80% power, a 1-sided hypothesis at 5% significance level for 3 variables: hemoglobin (Hb), serum ferritin (SF), and serum retinol. The SD for Hb in this population was 12.9 g/L (for both anemic and nonanemic girls) and 8.4 g/L (for only anemic girls) (30). Therefore, to detect a difference in mean Hb of 3.83 g/L between the MMB and UB groups required 141 girls per group for a nontargeted approach and 122 girls per group for only anemic girls. Based on an SD of 20.1 μg/L for SF from a previous study (76), 57 girls per group were required to detect a mean difference of 9.5 μg/L for SF between MMB and UB groups. Last, the SD of serum retinol from a previous study was 0.29 μmol/L; hence, 23 girls per group were required to detect a mean difference of 0.22 μg/L between MMB and UB groups. Expected mean differences for Hb (3.83 g/L), SF (9.4 μg/L), and serum retinol (0.11 μmol/L) are biologically plausible (43), which are within the range of our estimates. We considered the larger estimate ($n = 141$) of the 3 variables (Hb, SF, and retinol), and considering a maximum attrition rate of 10% during follow-up a minimum sample of 155 girls/group was considered for the RCT. With premenarche and postmenarche girls randomly assigned to the parallel arms of the RCT, the study had, in total, 4 groups, implying a total of 620 adolescent girls were required for the RCT (310 premenarche and 310 postmenarche).

Last, we estimated the sample size for the 24hR with the 1 random sample formula considering a 95% CI, an estimated width of 10.13 mg, and an SD of 28.9 mg for iron intake, as well as an estimated width of 50.5 μg retinol equivalents (RE) and an SD of 113.2 μg RE for vitamin A intake (77). For both iron and vitamin A intake, the estimated sample was 130 girls and, considering an attrition rate of 20%, this was rounded up to 150 girls for each menarche/age cohort. Using the rule of thumb recommended by Rothman (78), a subsample of

TABLE 1 Inclusion and exclusion criteria for Ten2Twenty-Ghana study[1]

Inclusion criteria for the survey	Exclusion criteria for RCT
Aged 10 to 17 y (verified by birth certificate, health record, insurance card, school register, or another formal document)	Severely anemic [Hb <80 g/L (79)]
Apparently healthy without any visible sign(s) of poor health	History of medical/surgical events that may significantly affect RCT outcomes
Nonpregnant	Sign(s) of chronic infection or metabolic disorder
Nonlactating	Clinical sign(s) of vitamin A deficiency and/or iodine deficiency
No incompatible mental status	Severely underweight (BAZ < −3 SDs)
Willing to participate	Taking medical drugs or nutrient supplements at the time of enrolment
Informed consent of parent or guardian obtained for survey	Participating in another food, supplement, and/or drug study
	Not willing to consume biscuits from Monday to Friday for 26 wk
	Any known food allergy to biscuits
	Afraid or not willing to donate ∼12 mL of blood on 2 different occasions
	Refusal of parents or guardian
	Second informed consent from parent or guardian obtained for RCT[2]

[1]BAZ, BMI-for-age *z* score; Hb, hemoglobin (those who were severely anemic were referred to a hospital); RCT, randomized controlled trial.
[2]Ethical approval requirements demanded that we obtain 2 different informed consents for the cross-sectional survey and the RCT.

50 per cohort from the first recalls was included in a repeated recall to allow for adjustment for random day-to-day variation in intake at the population level. Focus groups typically have 6–12 members plus a moderator (80), but Wyatt et al. (81) indicate that focus groups with 4–6 children are most effective in yielding valuable information because duplicate responses are less common and smaller groups are easier to control. According to Wyatt et al., children may be reluctant to talk in larger groups. Hence, we sampled 5 girls for the composition of each focus group in the survey and the RCT endline. Finally, Rothman's (78) rule of thumb was used to decide on 50 RCT-nonenrolled girls as a control group for the RCT-enrolled girls for the body-composition analysis.

Screening and sampling for the RCT

In Mion District, where the study was carried out, there are 70 primary and 11 junior high schools. The latter were excluded due to an already ongoing iron and folate supplementation project called GIFTS (Girls' Iron Folate Tablet Supplementation) among adolescent girls in junior high schools (82). While the Ghana Education Service (GES) has zoned Mion District into 6 clusters, for the Ten2Twenty-Ghana project 2 clusters were excluded based on their inaccessibility (remoteness). The remaining 4 clusters had 41 primary schools, and we ranked these schools in descending order based on the size of their female child enrollment using secondary data on enrollment obtained a priori from the GES in Mion. We purposively selected all the urban primary schools ($n = 4$) and the larger rural primary schools ($n = 15$) for screening until the minimum sample required for the survey ($n = 1040$) was met. In each school, we screened all the girls using a 16-item screening questionnaire including personal and household identification and demographics, menarche status, pregnancy and lactation status, health condition, use of any medical drug, iron supplements, and participation in a study with drugs, supplements, or food. Subsequently, girls who met the survey's inclusion criteria were invited to participate after obtaining their assent and their parents'/guardians' informed consent.

During the enrollment of subjects into the RCT, we added participants who were not postmenarche at in the survey using age >13 y, ensuring that we had enough sample size for randomization into the postmenarche group of the RCT. The cutoff age (>13 y) was chosen in conformity with the average age at menarche in Ghana from the literature (83–85). Thus, the postmenarche group in the present study includes postmenarche girls at screening or who were expected to become postmenarche during the RCT.

Probability proportion to size was used to select a random subsample of girls from the survey who did not meet the exclusion criteria of the RCT in 2 stages. First, we generated random numbers (between 0–1) by school and menarche group in Microsoft Excel (MS Excel, version 2016; Microsoft Corporation). The random numbers were sorted in ascending order (lowest to highest); the first set of participants from the menarche group of each school was enrolled until the sample size required for the school's menarche group was met. Any girl who dropped out during the enrollment was replaced with the next girl in the list from the same school and menarche group in the ascending order until the sample requirement was met. Subsequently, a second set of random (between 0–1) numbers was generated for the girls enrolled in the RCT in MS Excel. All enrolled subjects with random numbers <0.5 were assigned to a yellow color code, while subjects with random numbers ≥0.5 were assigned to a red color code.

The first step of the probability proportional to size approach described for the RCT was again used to randomly select 155 girls from each of the RCT menarche groups for the first 24hR. For the repeated 24hR, another random selection process like the preceding was used to select 100 girls from the sample for the first 24hR. At the RCT endline, the probability proportion to size approach was once more used to select 50 RCT-nonenrolled girls for body-composition assessment.

In-depth interviews are known to be labor intensive, and most studies utilizing in-depth interviews are based on <50 cases (80), explaining our decision to randomly sample 30 girls with at least 1 girl from each participating school. Additionally, in the extensive survey, we randomly

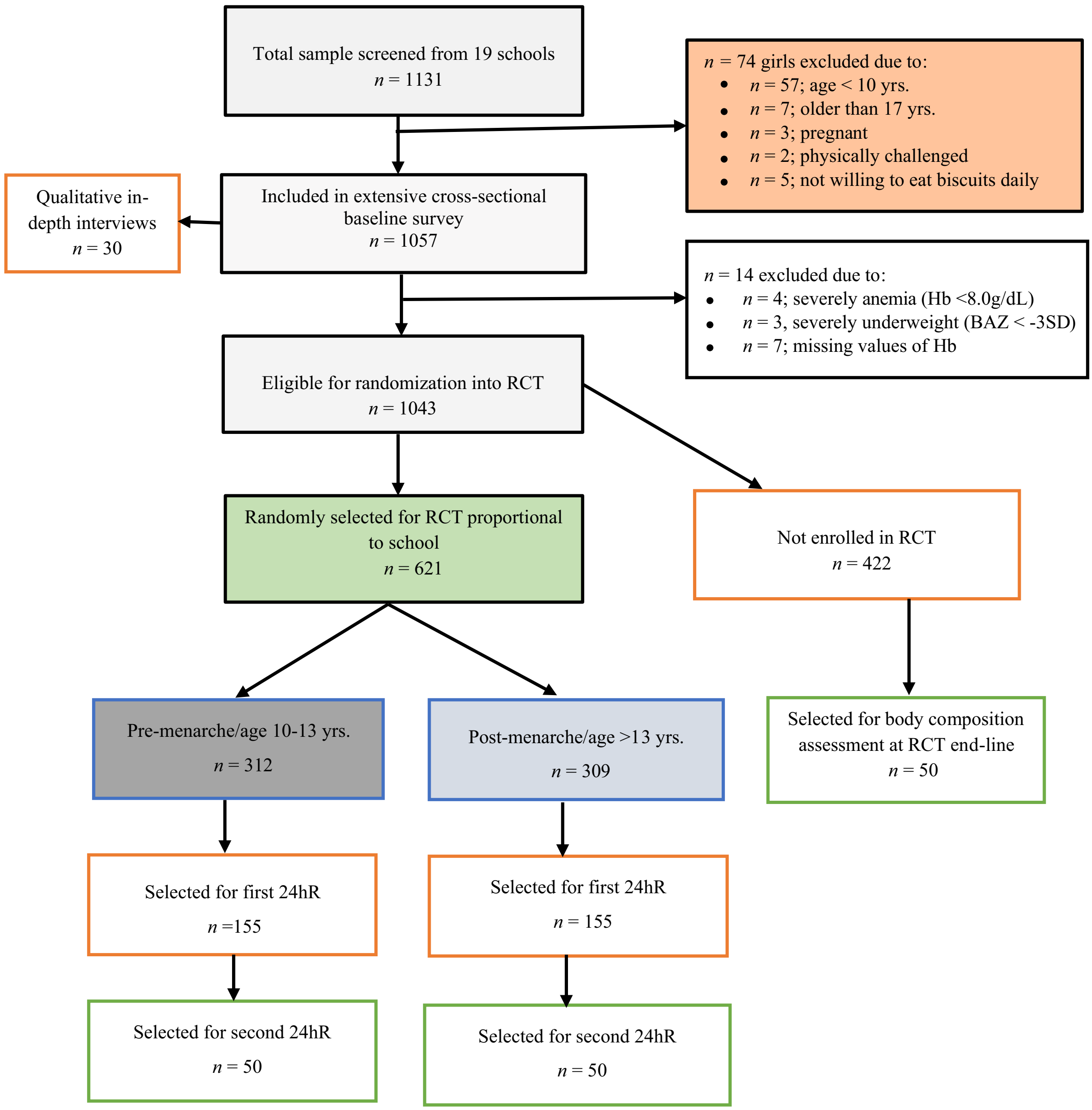

FIGURE 4 Flowchart for study population selection for the RCT and 24hR. BAZ, BMI-for-age *z* score; Hb, hemoglobin; RCT, randomized controlled trial; 24hR, quantitative 24-h dietary recall.

selected 2 clusters, and from each cluster 1 school was randomly selected for a focus group discussion. We next selected 5 girls randomly from each of the selected schools to compose the focus group. Last, we randomly selected 4 out of the 19 participating schools for 4 focus group discussions at the RCT endline. The 4 selected schools were next randomized for 1 focus group in each arm of the RCT (2 yellow groups and 2 red groups); the first 5 girls in each of the biscuit groups constituted the school's focus group. All of the randomizations were done using random-number generations in MS Excel. Figure 4 describes participant flow, as per CONSORT (Consolidated Standards of Reporting Trials) guidelines including reasons for nonenrollment in the RCT.

Run-in to the RCT

At the RCT baseline, all participants were dewormed against intestinal parasites with a single dose of mebendazole 400 mg chewable tablets. Malaria rapid diagnostic cassettes (First Response; Premier Medical) were used to screen for current or recent malaria. Participating girls who were found to have malaria during the run-in or the intervention period were treated promptly with artemether-lumefantrine (80 mg/480 mg) according to the guidelines of the Ghana Health Service (GHS) (86) and were referred to the local health facilities when necessary. We repeated the malaria screening and treatment at the midpoint and endline of the RCT. To assess the sensitivity and specificity of the malaria rapid test kits, we undertook malaria microscopy for ~11% (68 out of 621) of the girls at the midpoint malaria screening. During the run-in period (5 d), all enrolled subjects received UBs procured from the open market. The run-in biscuits' nutrient content was similar to the UBs (**Table 2**) in the RCT and was similar in size (50 g) to the MMBs and UBs for the RCT. The run-in was necessary for a priori data on the girls' compliance, the feeding set-up practice in each school, and the completion of a daily case-report form by the teachers and supervisors.

Biscuit formulation

van Stuijvenberg et al. (87) argued that biscuits are convenient food vehicles since they do not require any preparation by consumers, are relatively easy to distribute, and have a long shelf life. According to van Stuijvenberg et al. (87), biscuits are snacks rather than a meal and are unlikely to replace meals at home. Subjects in the treatment arm of the RCT received MMBs enriched with 11 vitamins (vitamins B-1, B-2, B-6, B-12, A, D, K-1, and E and niacin, folate, and ascorbic acid) and 7 minerals (zinc, calcium, iron, copper, iodine, selenium, and magnesium) as shown in Table 2. On the other hand, participants in the control arm received UBs that were similar in appearance to the MMBs. The UBs were simply wheat flour without any additional micronutrients. However, the wheat flour was fortified as by law in Ghana (Table 2). The average weight of a pack of each of the biscuits was 51.3 ± 3.2 g. A pack of each biscuit contained between 8 and 10 pieces of biscuits, with the average weight of a piece being 5.6 ± 0.5 g.

The micronutrient mix (fortificant) used for the fortification was procured from DSM Nutritional Products (South Africa), and the biscuits were produced by Mass Industries (Tema, Ghana) through the coordination of the Obaasima project. The Obaasima project is a scheme developed by the project "Affordable and Nutritious Foods for Women" (ANF4W), which is a partnership between the German Development Cooperation (GIZ) and the private sector in Ghana (http://obaasimaghana.com/campaign.php). Both biscuits (MMBs and UBs) provided 477.3 kcal per 100 g (244.85 kcal for the 51.3-g pack of biscuits) (Daniel Amanquah, Sight and Life; personal communication 2018) and hence varied only in micronutrient content (Table 2). We estimated the energy requirement of the girls using their mean bodyweight (35.8 ± 7.3 kg) and the FAO/WHO/UNU algorithms with the software Optifoood. Energy intake from the biscuits was then estimated to be ∼10% of the girls' required energy intake per day. We sent 3 packs of each of the biscuits to Wageningen University and Research (WUR; The Netherlands) for independent and confirmatory analysis of iron, calcium, and magnesium nutrient content. The packaged biscuits' shelf life was indicated to be 12 mo (Daniel Amanquah, Sight and Life; personal communication), and no organoleptic changes were expected during this period.

When received, the MMBs' and the UBs' original packages were distinguishable, so we re-packaged them to ensure blinding of the first author, the field team, and subjects. Both the MMBs and UBs were re-packaged in clear zip-locked bags with yellow and red stickers to distinguish between them 2 wk before the RCT baseline plasma sample collection. The re-packaging was done cautiously, opening the biscuit pack, and instantly pouring the entire contents into a zip-locked bag. **Figure 5** illustrates the original packaged and re-packaged biscuits. The re-packaging was done in an enclosed room in batches for 2–4 wk feeding and was coordinated by the project field supervisor from the UDS; he kept the seal to the color codes and was no longer blinded.

Administration of the biscuits

We recruited and trained 1 teacher from each school to administer the biscuits to the girls. Four trained field research assistants with a nutrition background were each assigned to a cluster of schools; they supervised the teachers and participated in at least 2 feeding sessions weekly in each school. Separate classrooms were used for the different biscuit colors during feeding sessions. The girls consumed the biscuits ad libitum as a snack during one of the school-break periods, Monday through Friday, in the teacher's and/or field assistant's presence. During the school holiday period, the girls, together with their teacher, agreed on a convenient time to come to school for the feeding. For girls who failed to turn up during a feeding session, the teacher and/or field assistant visited them to administer the biscuits. Girls could eat during the weekend (Saturday and Sunday) to make up for any lost day of feeding during the week. A maximum of 2 d lost in feeding during a week was allowed, and they could not carry forward a previous week's feeding to the next week.

Each girl was given a laminated sheet of her assigned color code (yellow or red) in bold letters to hand out to collect the biscuits each day. The teacher who supervised the daily feeding also had a daily case-report form containing the girls' list in each school's color code. The case-report form captured attendance, and leftovers (if any) were counted and recorded in the daily case-report form as pieces leftover. The daily case-report forms also captured any adverse events (AEs) and severe AEs (SAEs) during the feeding. The RCT management and supervision team included the field supervisor, the first author, and 4 trained research assistants recruited from the UDS. Each research assistant was assigned to supervise a cluster of schools (**Figure 6**).

Use of Co-intervention

As biscuits are generally dry to consume, 500 mL filtered and packaged sachet water produced by the Nyankpala Campus of the UDS was provided daily alongside the girls' biscuits to help wash down the biscuits. Additionally, nutrition and health education was provided to all students in the selected schools. The educational component included modules on anemia, dietary diversification, personal and environmental hygiene and sanitation, malaria in Ghana, and sexual and reproductive health education. The nutrition/health education was provided as a complement to improve the girls' awareness and knowledge about their health, nutrition, and reproductive health. Subjects received the nutrition and health education modules (**Table 3**) on 5 different occasions for the entire duration of the study through lectures, group discussions, and demonstrations; each session lasted ∼1 h. Color picture aids were used to aid the sensitization. The sensitization was conducted by community health nurses who stay and work in each of the communities. The nurses were recruited and given a 1-d training with the district health advocacy team directly supervising them.

Data-collection methods

We used a mixed-methods data-collection technique applying both quantitative and qualitative data-collection methods for triangulation in the study. A pretested questionnaire was used to collect data on various social, economic, and health-related topics to provide comprehensive information on the girls' social and economic trajectories. The questionnaire was pretested in a pilot survey, in November 2017, in the neighboring Yendi municipality. The data-collection methods included

TABLE 2 Nutrient content of biscuits for the Ten2Twenty-Ghana RCT[1]

No.	Nutrient	Product name	Nutrient content of fortified biscuits per serving (51.3 g), mg	Nutrient content of unfortified biscuits per serving (51.3 g),[2] mg
1	Vitamin A	Dry vitamin A palmitate	613.72	0.10
2	Vitamin D	Dry vitamin D-3	134.00	0.00
3	Vitamin E	Dry vitamin E	6.00	0.00
4	Vitamin K	Dry vitamin K-1	0.05	0.00
5	Thiamin	Thiamin mononitrate	1.20	0.43
6	Riboflavin	Riboflavin	1.20	0.23
7	Niacin	Niacinamide	14.00	3.03
8	Vitamin B-6	Pyridoxine hydrochloride	1.60	0.00
9	Folic acid	Folic acid	0.311	0.11
10	Vitamin B-12	Vitamin B-12	0.002	0.001
11	Ascorbic acid	Ascorbic acid	70.00	0.00
12	Calcium	Calcium carbonate	150	0.00
13	Copper	Copper gluconate	0.20	0.00
14	Iodine	Potassium iodide	0.04	0.00
15	Iron	Ferrous fumarate	4.05	1.03
16	Magnesium	Magnesium oxide	52.50	0.00
17	Selenium	Sodium selenite	0.012	0.00
18	Zinc	Zinc oxide	2.38	1.45

[1]RCT, randomized controlled trial.
[2]Obtained from the laboratory division of Mass Industries, Tema-Ghana; the nutrient content reflects the fortification level of wheat flour in Ghana by law.

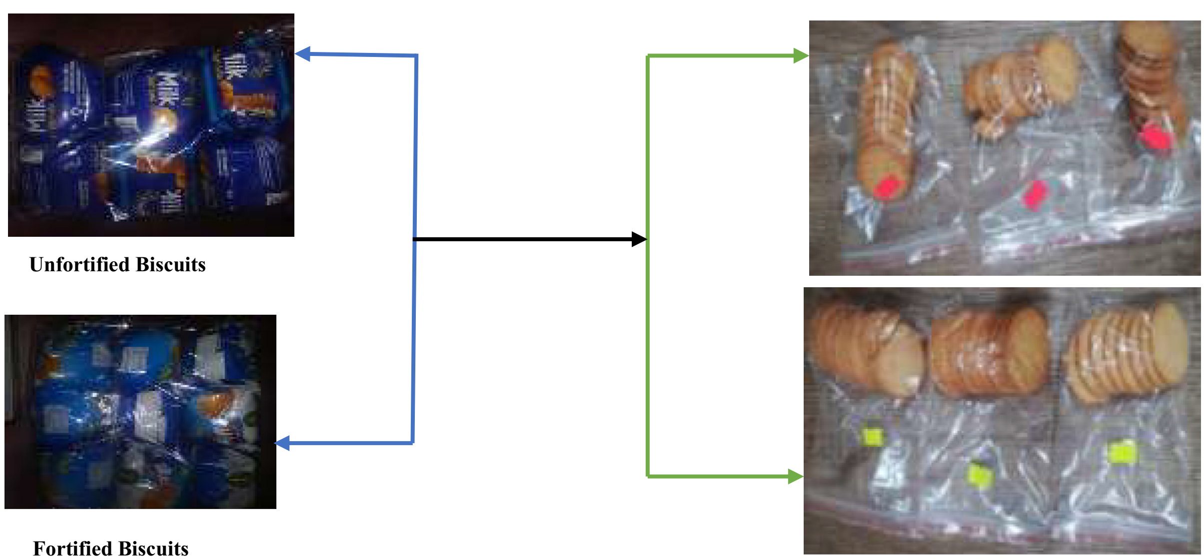

FIGURE 5 Repackaged biscuits for the Ten2Twenty-Ghana RCT. RCT, randomized controlled trial.

one-on-one interviews, anthropometry, Hb status assessment, the collection of venous blood for plasma, a quantitative 24hR, in-depth interviews, and focus group discussions. Table 4 shows a summary of the data collected in the extensive survey and RCT. We first describe the data collected in only the RCT and then data collected in both the extensive survey and/or RCT at endline.

Data collected in only the RCT

Except for venous blood and the quantitative 24hR, data from the larger survey informed the baseline data of subjects enrolled in the RCT, including Hb, the secondary outcomes, and covariates. The RCT data are grouped into primary outcome data, secondary outcome, data and covariates (Table 5).

Plasma samples.

Hb assessment for the survey was by finger prick using a HemoCue 301 (0.1g/dL precision) 2 mo preceding the RCT. At baseline and endpoint of the RCT, a phlebotomist from the Tamale Teaching Hospital (TTH) collected ∼10 mL venous blood (nonfasting state) from each subject for biomarkers of micronutrient status into 2 (4 mL each) Na-Heparin Vacutainers (Becton-Dickinson Diagnostics). The biomarkers being assessed include plasma SF, soluble transferrin receptor

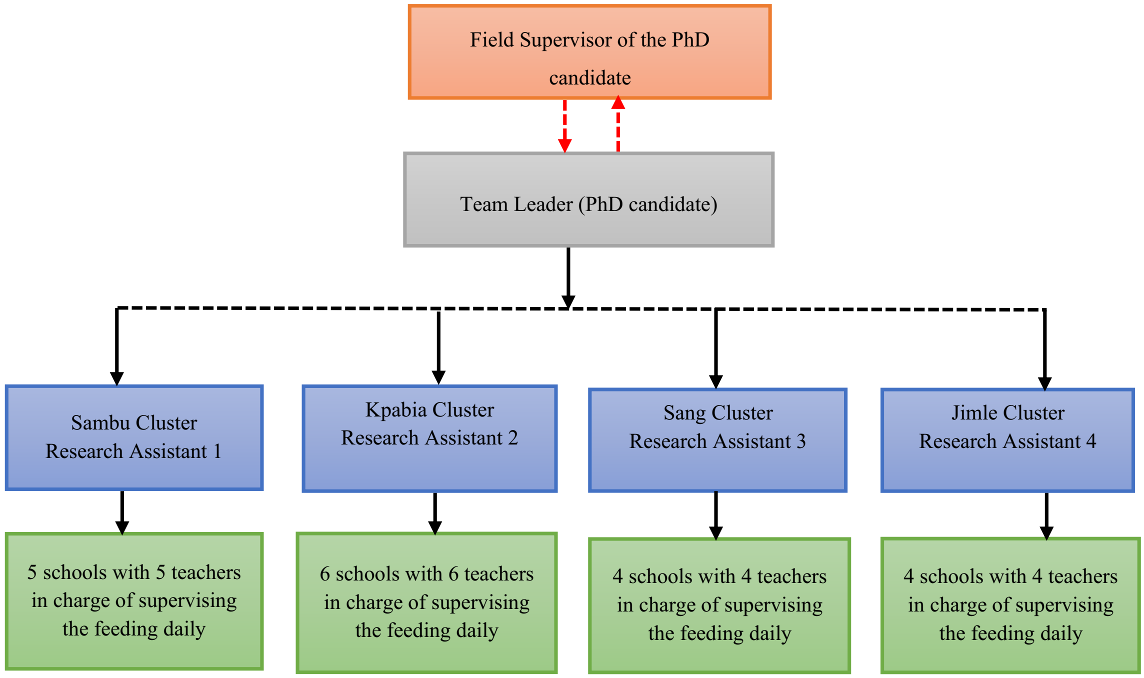

FIGURE 6 Field supervision plan in the Ten2Twenty-Ghana RCT project. RCT, randomized controlled trial.

concentration (TfR), retinol-binding protein (RBP), C-reactive protein (CRP), and α-glycoprotein (AGP), zinc, folate, and vitamin B-12. At the RCT endline, we assessed Hb status in the field using a small portion (∼2 mL) of the venous blood with the HemoCue 301. The blood samples were kept in a cool opaque box containing freezer packs (∼0°C) in the field and during transport from the field. The venous blood was centrifuged in Rotofix 32A centrifuge at 4000 rpm for 5 min at the end of each field day at room temperature. The centrifuging was done at the emergency services laboratory of the TTH. We pipetted and stored 2.5 mL plasma in duplicate 1.25-mL cryptogenic vials at −20°C (Thermo Fisher Scientific) at the Public Health Laboratory of the TTH, Ghana.

Plasma samples were subsequently transported 2 mo after the RCT on dry ice to WUR for storage in liquid nitrogen gas (−88°C). One-hundred microliters (100 μL) of the plasma samples were then pipetted into Micronic tubes and shipped on dry ice to the VitMin Lab (Willstätt, Germany) for the analysis of SF, TfR, RBP, CRP, and AGP using a combined sandwich ELISA technique (88). All measurements were done in duplicate, and where the CV (interassay) was >10%, measurements were repeated and obvious outliers removed. The CVs for the various indicators were as follows: SF, 2.3%; TfR, 3.6%; RBP, 3.6%; CRP, 5.8%; and AGP, 8.1%. Certified quality-control samples from the CDC/Atlanta and Bio-Rad Liquicheck controls (Bio-Rad) were used to calibrate the

TABLE 3 Modules for nutrition and health education in the Ten2Twenty-Ghana RCT[1]

Module 1: Water, Hygiene, and Sanitation (WASH)	Module 2: Anemia, Malaria, and Dietary Practices	Module 3: Sexual and Reproductive Health Education Part 1	Model 4: Sexual and Reproductive Health Education Part II	Recap of all modules
Food and water hygiene	Anemia: causes, signs/symptoms, and prevention	Menstruation and menstrual hygiene	Sexually transmitted diseases (STDs): types, causes, and prevention	A re-cap of all topics discussed; group discussions and questions-and-answers session
Household and environmental hygiene	Malaria: causes, symptoms, consequences, and prevention	Sexual behavior		
Personal hygiene and good grooming	Healthy dietary practices for children and adolescents	Teenage pregnancy: causes and consequences		

[1]RCT, randomized controlled trial.

TABLE 4 Details of the data collected in the Ten2Twenty-Ghana research project[1]

Data	Data collection period		
	Survey (November/December 2018)	RCT baseline (January–March 2019)	RCT endline (September 2019)
n	1057	621	588
Individual characteristics			
Age(date of birth)	√	—	—
Birth order	√	—	—
Girl's education	√	—	—
Religion	√	—	—
Ethnicity	√	—	—
Maternal data			
Anthropometry of the biological mother	√	—	—
Final decision-making index	√	—	—
Fertility and labor history calendar	√	—	—
Household characteristics			
Parental education and occupation	√	—	—
HH rooster (sex, age structure, religion, education, occupation, and literacy)	√	—	—
HH wealth index (International Wealth Index)	√	—	—
Psychosocial outcomes			
Self-reported HRQoL	√	—	√
Subjective health complaints	√	—	√
Life satisfaction	√	—	√
Self-esteem	√	—	√
Self-efficacy	√	—	√
Body image	√	—	
Children's Depression Inventory	—	—	√
Cognitive skills and academic performance			
NIH toolbox for cognition	√	—	√
Secondary data on academic performance and school attendance[2]	√	—	√
Reproductive health and sexuality			
Age at menarche (recall)	√	—	√
8-item PDS	√	—	√
Relationship (boyfriend)	√	—	—
Age at first sex (if applicable)	√	—	—
Marital status	√	—	—
Age at marriage if married or ever married	√	—	—
Number of biological children if any	√	—	—
Age at first birth (if any)	√	—	—
Dietary intake and nutritional status			
Dietary Diversity Score (single qualitative 24hR)	√	—	√
Household food security (FIES)	√	—	√
One-month FFQ	√	—	√
Frequency of the consumption of energy drinks	—	—	√
Quantitative 24hR repeated with a subsample on nonconsecutive days (USDA standard multiple-pass procedure)	—	√	—
Anthropometry	√	—	√
Body composition (bioelectrical impedance)	√	—	√
Biomarkers of nutritional status			
Hb (HemoCue)	√	—	√
Plasma micronutrient status (ferritin, TfR, RBP, zinc, folate)	—	√	√
Inflammation biomarkers (CRP and AGP)	—	√	√
Qualitative data collection			
Focus group	√	—	√
In-depth interviews	√	—	—

[1]AGP, α-glycoprotein; CRP, C-reactive protein; FFQ, food-frequency questionnaire; FIES, Food Insecurity Experience Scale; Hb, hemoglobin; HH, household; HRQoL, health-related quality of life; PDS, Pubertal Development Scale; RBP, retinol-binding protein; RCT, randomized controlled trial; TfR, plasma transferrin receptor; 24hR, quantitative 24-h dietary recall.
[2]The data were collected for the overall sample from the cross-sectional survey (*n* = 1057) at both time points.

TABLE 5 Outcomes and covariates assessed in the Ten2Twenty-Ghana RCT[1]

Primary outcomes	Secondary outcomes	Covariates
Changes and difference in micronutrient status between biscuit groups in:	Changes and differences between biscuit groups in anthropometric indicators (e.g., attained height, height-for-age *z* score, BMI-for-age *z* score), and body composition (fat mass, fat-free mass, muscle mass, skeletal muscle mass, body cell mass, total body water, extracellular water, and intracellular water)	Inflammation biomarkers (C-reactive protein and α-glycoprotein)
■ Hb status	Changes and differences between biscuit groups in cognitive skills and academic performance, perceptions, and aspirations (qualitative)	Dietary diversity score, dietary patterns, household food security
■ Plasma SF		
■ Plasma soluble TfR concentration		
■ RBP		
■ Plasma zinc		
■ Plasma folate		
■ Vitamin B-12		
Quantitative dietary intake for a subset ($n = 310$)		
		Demographics (age, education, religion, ethnicity, household composition) and socioeconomic covariates (household wealth index, parental occupation and education)
	Changes and differences between biscuit groups in psychosocial health and competencies such as health-related quality of life, self-efficacy, self-esteem, life satisfaction and subjective health complains	

[1]Hb, hemoglobin; RBP, retinol-binding protein; RCT, randomized controlled trial; SF, serum ferritin.

results. Plasma samples from the 2 time points (RCT baseline and endline) were analyzed at the same time. Plasma zinc was analyzed with atomic absorption spectrometer, while folate and vitamin B-12 were analyzed with HPLC later.

Quantitative 24hR.

We assessed the current intake of a subsample of the girls enrolled in the RCT with a quantitative 24hR using the USDA standard multiple-pass procedure (89). To enable adjustment for random day-to-day variation in dietary intake, we repeated the quantitative 24hR in a subsample ($n = 100$) of the girls with a first quantitative 24hR on nonconsecutive days to avoid dependency of intake. Trained interviewers conducted the dietary interviews at home, 1 mo preceding the RCT. We randomly assigned subjects to all days of the week and interviewers to account for differences in intake between days and interviewers. No interviewer could interview the same subject twice.

In the standard multiple-pass procedure, the girl was first asked to mention all foods and drinks, including snacks that she consumed in and outside the home (including school) the previous day. She was then asked to describe the ingredients and cooking methods of any mixed dishes. The primary caregiver and/or the person who prepared home meals the preceding day was asked to help the girl list and estimate ingredients for mixed dishes prepared at home. We recorded the actual weight of a duplicate portion of each food, beverage, and ingredients of mixed dishes using a digital kitchen scale (Soehnle Plateau, model 65086) precisely to 2 g with a maximum capacity of 10 kg. In the absence of duplicate portions in the household, amounts were estimated as their monetary value equivalents, weight-to-weight with other foods (e.g., amount of sugar estimated with refined corn flour), in volumes, food models (small, medium, or large), or as household units in priority order. The research team agreed a priori on models for food such as onion, tomatoes, and garden eggs, which were carried alongside. We estimated the total volume of each mixed dish cooked at the respondent's household and the volume of this dish consumed explicitly by the girl to determine the proportion of the dish she consumed. The amount of ingredients consumed from mixed dishes by the girl was estimated by multiplying the proportion consumed with the total amount of ingredients used to prepare the dish. We also recorded each food ingredient's frequency of intake (for mixed dishes) or food item for the last 7 d preceding the interview day. For shared-bowl eating, the girl's usual intake for such dishes and the number of persons who ate from the shared bowl were recorded in the logbook. The 24hR ended by probing the girl for likely forgotten foods—notably, fruits, sweets, and snacks consumed on the recall day.

Standard recipes and school feeding recipes were generated to estimate grams of ingredients consumed from mixed dishes eaten outside the home or from the school feeding program. Estimates of these recipes were obtained by averaging 3 recipes of different vendors and different school feeding matrons/cooks. The vendors and school feeding matrons/cooks were each selected from different localities and schools.

Moreover, we developed conversion factors to convert monetary values, weight–weight measures, volumes, food models, and household units to their weight (grams) equivalents following Gibson and Ferguson (89). Last, we conducted a market survey in 4 different markets in each of the study clusters. We determined the mean price per 100 g of edible food for each listed food in the 24hRs using the average price and weight of foods obtained from each of the surveyed markets.

Data collected in the extensive survey and/or at RCT endline

Anthropometry and body composition.

Height and weight were measured in duplicates to the nearest 0.1 decimal with the Seca stadiometer and digital weighing scale, respectively, in the survey and at the RCT endline. Standard anthropometry guidelines were followed (90) in the assessment. Height and weight were transformed into height increment, attained height, *z* scores (height-for-age, BMI-for-age), and BMI. The *z* scores will be computed with the WHO AnthroPlus software with the WHO growth reference for adolescent girls aged 10–19 y.

In the survey and at RCT endline, we also assessed body composition with bioelectric impedance using the Bodygram Plus Analyser (Akern, Germany) (91). In the body-composition assessment, subjects laid in a backward position with their arms by their side on a field camp bed for 3–5 min to ensure uniform distribution of body fluids before the assessment. The girls' feet and wrist were wiped with nonalcoholic wipes before placement of the bioelectric electrodes for the appraisal. The electrical resistance (*Rz*) of the tissues and capacitive resistance of the cell membranes (XC) in whole numbers were recorded on a form and later input into Bodygram Plus Analyser's software(Akern, Germany) for the computation of body composition. Body-composition estimates were to the nearest 0.1 decimal. They included fat mass (kilograms), fat-free mass (kilograms), muscle mass (kilograms), skeletal muscle mass (kilograms), body cell mass (kilograms), total body water (liters), extracellular water (liters), and intracellular water (liters). The program also computes indices and percentage to the total body weight for these estimates.

Food security and other dietary data.

In the survey and at RCT endline**,** we assessed the girls' dietary patterns with a qualitative 1-mo food-frequency questionnaire (FFQ). A 10-food-group indicator (92) was adopted for the FFQ. Likewise, we assessed the frequency of the consumption of energy drinks using a list of energy drinks a priori collected through a market survey (Abdul-Razak Abizari, University for Development Studies; unpublished data 2018) at the RCT endline. A single qualitative 24hR was also used to assess the Dietary Diversity Score (DDS) of the girls using the 10-food-group indicator (92) in the survey and at RCT endline. Furthermore, the girls' households' food-security status was assessed with the Food Insecurity Experience Scale (93).

Fertility and marriage.

In the survey and at RCT endline, age at menarche was assessed by recall and pubertal development stage by a 5-item Pubertal Development Scale questionnaire (94, 95). A semi-structured questionnaire assessed relationships (sexual) of the girls.

Psychosocial outcomes.

Psychosocial health outcomes were assessed in the survey and at RCT endline with validated scales including self-reported health-related quality of life (HRQoL) using KIDSCREEN-27 (96, 97), subjective health complaints (98), self-esteem (99), self-efficacy (100), and life satisfaction (98, 101). Furthermore, the assessment included body image of subjects using the Stunkard figure rating scale (102) in the survey. Finally, we included and assessed depression using the Children's Depression Inventory (103) at the RCT endline.

Cognitive skills and academic performance.

The data included secondary data collected from the schools on the school attendance of the girls and of their grades in English Language, Mathematics, and General Sciences in the academic year prior to the study (September 2017 to July 2018) and at the end of the RCT (September 2018 to July 2019). The academic data were collected for the overall sample ($n = 1057$) from the survey at both time points.

At both time points (survey and RCT endline), we assessed the cognitive function of the girls with the NIH toolbox cognition battery (NIH-TCB) (104, 105). The NIH-TCB is a recognized and standardized test tool for measuring cognitive function. The test is computerized on iPads (Apple), and the scores are automated at the end of each test. The tests appeared as games the girls had to play, but since our subjects were generally from a rural setting, we recognized that they might be limited in playing computer games. Hence, they could point to the right answer on the screen, with the interviewer clicking for them instead. We assessed 5 domains of cognitive function, which were found based on the literature to be relevant to adolescents' neurological development (106, 107). The 5 domains included episodic memory with the Picture Sequence Memory Test, working memory with the List Sorting Working Memory Test, attention with the Flanker Inhibitory Control Attention Test, processing speed with Pattern Comparison Processing Speed Test, and executive/shifting function with the dimensional Change Card Sort Test. A set of unscored trial tests preceded the actual tests; the unscored test allowed the girls to practice before the actual test.

Labor, time use, and aspirations.

In the survey, we adopted the "Young Lives" questionnaire (68, 108) to assess the time use, labor participation, and earnings of the girls. A life history calendar (109) mapped the labor participation of the girls. Last, the questionnaire included the girls' expectations and aspirations for marriage, family formation, education, and work.

Maternal and household-related covariates.

In the survey, the anthropometric assessment included the height and weight (nearest 0.1 decimal) of the girls' mothers for whom BMI and maternal height would be used. The data also included the mothers' participation in household decision making using the Demographic and Health Survey 8-item final decision-making index (110). Life history calendars (109) also captured data on the mothers' fertility and labor participation.

Moreover, we enumerated household members with a household rooster including their sex, relationship to the index girl, age group, education, occupation, and literacy, ensuring that we can compute various household-related indices. Finally, the International Wealth Index (111) captured data on the household's socioeconomic status.

Focus group discussions.

In the survey and at RCT endline, focus group discussions were conducted by 2 trained research assistants who had previous experience with focus groups. They were trained to probe, listen, and record in writing as well as using a digital recorder the expressions of the girls. One of them moderated the discussions while the other recorded the discussions both digitally and in a notebook. Topics for discussion included the knowledge, attitudes, and practices (KAP) of the girls regarding relationships, reproductive health, risk behaviors, and dietary habits. The discussions also delved into the aspirations, expectations, and life satisfaction of the girls. In the survey, the focus groups also explored the KAP of the girls with regard to their body image. A visual storytelling technique was incorporated into the focus group discussions. The girls' data generated in the focus group discussions included digital records, notes, and worksheets used for sketches.

In-depth interviews.

According to Mack et al. (112), in-depth interviews are optimal for collecting data on individuals' personal histories, perspectives, and experiences, mainly when sensitive topics are being explored. Boyce and Neale (113) also posited that the approach provides detailed information about a person's thoughts and behaviors and in exploring new issues in-depth. We used in-depth interviews to explore rich insights into the girls' lives and understand their motivations, expectations, aspirations for the future, their life satisfaction, relationships, risk behaviors, and the challenges confronting them in their everyday lives. We also solicited information on their usual dietary patterns and the reasons for adherence to these dietary patterns.

Internal validity of the data

Several measures were taken to ensure the internal validity of the data. We recruited and trained field research assistants as well as supervisors with relevant field experience who could speak at least 1 of the key local dialects (Dagbani or Likpakpa) fluently. The training included 5 d for the survey, 3 d for the 24hR, 1 d for the focus group discussion, and a 3-d refresher for the endline. Due to the sensitive nature of questions regarding menarche, relationships, and sexuality, all interviewers administering the one-to-one questionnaire were women recruited from the UDS. In the field, supervisors checked and validated all questionnaires for consistency and completeness. A Microsoft Access template was designed and used for the data entry. The data template was coded numerically, such that implausible values in coded categorical data were impossible. All data entry clerks received a 5-d training by an Information, Communication Technology expert who oversaw the data entry. The entries were merged into a single MS-Access file and the data exported into different SPSS templates based on data themes. Data cleaning was performed in the SPSS templates in the field. The entries of 449 out of 1057 (42.5%) and 202 out of 589 (34.3%) questionnaires were verified entirely in all of the data files in the survey and at RCT endline respectively.

Statistical analysis plan

Data analysis will be conducted with SAS 9.4 (SAS Institute, Inc.) and IBM SPSS (version 25), where necessary. Frequencies and percentages are used to describe baseline summary statistics for categorical data while means $\pm$ SDs will be used for continuous, normally distributed data. Skewed continuous data will be presented as medians and IQRs. Data normality will be visually explored with histograms with normality curves, boxplots, and Q-Q plots. Baseline differences in proportions between biscuit groups will be determined with chi-square or Fisher's exact test, as appropriate. One-factor ANOVA or its nonparametric version (Mann-Whitney U test) will be used to determine differences in means between biscuit groups for descriptive population statistics. Summary statistics will be presented for sociodemographic, anthropometric, and micronutrient indicators at baseline by biscuit group in the RCT to describe the study population.

The RCT data analysis follows the intention-to-treat approach with a sensitivity analysis following per protocol (compliance $\geq$80%). Compliance is defined by the amount (grams) of biscuits consumed expressed as a percentage of the expected total amount that should have been consumed for the entire RCT. The effects of the fortified biscuits on micronutrient status will be analyzed using linear mixed models (LMMs) with maximum-likelihood estimations. LMMs are more robust in handling unbalanced and missing data; the models are also better able to handle the assumption of independence and homogeneity of slopes in the data (114). As our study population was selected from 4 clusters, 19 different schools, and different classes, LMM analysis is preferred over ANCOVA to adjust random hierarchical variables related to the cluster, school, and class of the girls. Similarly, we will use LMM analysis to examine the intervention's effect on cognition, body composition, and the psychosocial health outcomes (HRQoL, self-efficacy, self-esteem, and life satisfaction) of the subjects. A "Step-up strategy" (115) will be used in building the LMMs. The analysis of body composition includes the effect of the fortified compared with the unfortified biscuits, as well as the effect of being enrolled and not enrolled in the RCT. However, for dichotomous/categorical outcome variables, Cox proportional hazard models will be used to examine the incidence rate and prevalence risk ratio. Cox and Poisson models with robust variance are reportedly better alternatives than logistic regression (116, 117). We hypothesize that the fortified biscuits would significantly affect micronutrient status and the secondary outcomes; hence, a 1-sided hypothesis at 5% significance and 95% CI will be used in the analysis. We will adjust for a set of identified sociodemographic and socioeconomic confounding variables in all associations in the statistical analysis. A confounder will be defined as any variable that differs significantly between the biscuit groups at baseline or any variable contributing at least a 10% change in the crude effect estimates after adjustment (78, 118). All missing data will be imputed if $>$5% of data are missing using multiple imputation methods in SAS, assuming that the data are missing at random (119). Although no interim analysis was planned, the decision to conduct interim analysis was dependent on reports from the field on AEs and SAEs. The data safety monitoring committee had access to reports of the AEs and SAEs and could request for an interim report.

Plan for analysis of quantitative 24hR data

Compl-eat software (www.compleat.nl) of WUR will be used to estimate individual nutrient intake. Nutrient intake will be adjusted for random day-to-day variation in intake using the Statistical Program to Assess Dietary Exposure (SPADE) (120). To determine the population at risk of nutrient inadequacy, we will use the harmonized average requirements proposed by Allen et al. (121). Optifood linear programming (122, 123) will be used to develop and evaluate affordable alternative FBDGs that can fulfill or best meet adolescent girls' nutrient requirements. Pubertal timing may influence dietary habits/patterns of adolescent girls. For instance, mid-adolescent, compared with early adolescent, girls consumed fewer protein- and vitamin-rich foods in India (124). In addition, the nutrient requirements, notably iron for postmenarche girls, are higher than in premenarche girls. Hence, in the formulation of the FBDGs, stratified analysis by menarche status will be conducted. Last, principal components analysis will be used to identify dietary patterns of the girls.

Analysis of qualitative data

We will use the inductive thematic analysis approach (125) in analyzing all qualitative data from the in-depth interviews and focus group discussions. The analyses will focus on the similarities and differences in the themes within transcripts. This method provides a rich and detailed account of data and the themes emerging from the data (126). Analyses include transcriptions of digitally recorded discussions, field notes, and worksheets from the girls in the focus groups. We will conduct open-ended coding on each text unit (e.g., sentence or paragraphs) and coding the "raw" participant data, such as quotes. The different categories will be sorted into potential themes and all of the relevant coded data extracts will be collected within the identified themes. Coding and categorizing will be done using ATLAS ti (version 8.0; Scientific Software Development) data-management software, which will facilitate the retrieval of coded chunks of transcripts.

Ethics approval and consent to participate

The protocol was approved in January 2019 by the Navrongo Health Research Centre Institutional Review Board (NHRCIRB323). The RCT was prospectively registered with the Netherlands Trials Register (https://www.trialregister.nl/trial/7487) with registration number NL7487 in February 2019. A data safety monitoring committee comprised of 3 independent persons with relevant experience in nutrition trials reviewed the trial's safety monthly during implementation. Before the study, a stakeholder meeting was held with the Mion District Assembly, the GES and GHS, and all heads of the selected schools in the district capital Sang. Written permission was next obtained from the GES in the district. We also undertook a community entry sensitization with all of the opinion leaders, the School Management Committee, the Parent-Teacher Association, and teachers of all the selected schools. Last, in the survey and RCT, we invited parents of the eligible girls for sensitization and education about the study at the school; their signed/thumbprinted informed consent for their female child's participation was then sought. Data collected remain confidential, and study results will be reported in aggregated form so that participants remain anonymous. Only members of the RCT team had access to participants' records. RCT assistants also signed a written statement to maintain the confidentiality of any personal information from trial participants with whom they may become acquainted.

Discussion

We designed an innovative mixed-methods study entitled "Ten2Twenty-Ghana," starting with an extensive survey leading to a 26-wk RCT. The study's overall aim is to evaluate the efficacy of consuming MMBs compared with UBs for 5 d/wk for 26 wk on micronutrient status, vertical growth, body composition, cognition, psychosocial health, and fertility of adolescent girls. We also aim to examine how the intervention effect relates to the intervention's timing (before or after menarche) and formulate and evaluate affordable, evidence-based, alternative FBDGs best to fulfill the adolescent girls' nutrient requirements.

Overall, it is expected that the girls' micronutrient status in the fortified biscuit arm of the trial will be improved alongside improvements in their vertical growth, cognitive development, and psychosocial health in the long term. Das et al. (127), in a systematic review and meta-analysis, showed that food fortification with vitamin A, iron, and multiple micronutrients for children significantly increased hematologic biomarkers and serum micronutrient concentrations. In our research design, Hb and micronutrient status are primary outcomes for which we hypothesize significant improvement for girls receiving the MMBs compared with those receiving the UBs.

Micronutrient deficiencies often coexist, and micronutrients interact with each other. Accordingly, multiple-micronutrient interventions may be more effective in improving nutritional status (53, 128), informing our decision to use multiple-micronutrient fortification. However, in the RCT design, we selected micronutrient biomarkers known to be of public health significance to SSA adolescents based on the literature for assessment (27, 28, 127). Retinol remains the recommended biomarker for assessing the vitamin A status of populations. However, the analysis of RBP is relatively easier, and RBP when combined with CRP has been shown to produce an unbiased estimate of vitamin A deficiency (VAD) in a setting such as ours (129). Further, Larson et al. (130) illustrated that the internal regression correction approach we would use accounts for the severity of inflammation when estimating VAD prevalence in regions with high inflammation and malaria.

Indeed, longer-term consumption of fortified foods may be more beneficial. However, based on a review of comparable interventions (41, 43), we anticipate that 6 mo will suffice to at least observe a positive trend between improved micronutrient status and the secondary outcomes including cognition, vertical growth, fertility, and psychosocial health. In a group of Bangladeshi adolescent girls, Hyder et al. (43) found significant increases in weight, mid-upper arm circumference, and BMI over 6 mo for the fortified group compared with the unfortified group ($P = 0.01$). A 6-mo trial by Wang et al. (41) in Chinese adolescents illustrated that fortified-food consumption improves academic performance, motivation, and learning strategies.

Overall, the biscuits' energy was expected to help sustain the girls in school and may improve the weight and school attendance for all participants in the RCT compared with those not enrolled. To better understand this, we assessed the body composition of 50 girls not enrolled in the RCT. We collected secondary data on all of the girls' academic performance and school attendance from the survey ($n = 1057$) at the RCT endline. Our approach allows us to conduct a comparative analysis between those who benefited from the RCT as compared with those who did not, for academic performance, school attendance, and body composition. Although our study design does not evaluate the nutrition education component, we expect improved awareness about nutrition and health, including sexual and reproductive health.

There were no foreseen risks to participants following their consumption of the MMBs. Like the MMBs used for the trial, fortified foods have previously been used in efficacy trials without any SAEs. Although iron is often associated with some side effects, the 4.05 mg Fe added to the biscuits is within the recommended dose for supplementation and fortification (131, 132). Even at a higher dose of 1300 mg daily among iron-deficient American adolescent schoolgirls, the only side effect that differed significantly between the iron-supplemented and placebo groups was stool color (133), suggesting that the risk of any side effects would be much lower among girls with poor micronutrient status. Although vitamin A is toxic when ingested in large quantities, none of the studies using MMFs have reported any side effect associated with vitamin A intake.

To our knowledge, limited research has focused systematically on girls' transition into adulthood or acknowledged the interplay of different and parallel life trajectories. Hence, the present study would contribute to knowledge on the interaction of varying life trajectories on girls' nutrition and health in a context such as Ghana. Furthermore, our study design allows us to determine the efficacy and optimal timing of an MMF program for adolescent girls. The study would enhance our current understanding of the extent of micronutrient deficiencies such as iron and IDA and vitamin A among adolescent girls. In the more extensive survey, we attempt to explore the interrelations between the girls' nutrition and their labor participation, earnings, aspirations for work, education, and family formation. Although we cannot examine any causal associations, our data ensure that we can describe some associations between maternally related factors and the girls' nutrition and health.

This study also produces data on the nutrient gaps in adolescent girls' diets in a developing context. Finally, our research will conclude by developing evidence-based FBDGs to best meet or fulfill adolescent girls' nutrient requirements in a rural Ghanaian setting. Such data are urgently needed to help build common ground among program planners and implementors to include MMFs in intervention programs designed to improve adolescent girls' health in developing contexts. Such MMF programs may be vital to breaking the vicious cycle of intergenerational malnutrition in the long term.

Strengths and limitations of the study

Most of the postmenarche girls were found in junior high schools, which we excluded due to the GIFTS program (82), jointly implemented by the GHS and UNICEF. To overcome this challenge, we included 6 more communities and schools in the study population, leading to the 19 schools (instead of 13 schools estimated a priori) involved in the present study. We also included girls who were expected to become postmenarche during the RCT with reference to the average age at menarche in Ghana (83–85).

There was a 2-mo lag period between the extensive survey and the baseline plasma sample for the RCT. The lag period was unplanned and related to a delay in our receipt of the biscuits, reflecting the logistic challenges in conducting a study in rural Ghana. However, any possible bias emerging is random and equally distributed in the MMB and UB groups in our RCT, and therefore unlikely to affect our results. Further, a 2-mo period may be a short time to cause any significant change in the secondary outcomes and covariates of the RCT baseline, informed by the extensive survey data.

Hb is higher in capillary blood than in finger-prick compared with venous blood because venous blood is deoxygenated (134). In the present study, this would have resulted in a systematic overestimation of the baseline Hb, which was by finger prick, compared with the endline Hb, which was by venous blood. However, since the bias was systematic across groups, it would have little influence on the MMB and UB postintervention differences.

To the best of our knowledge, this is the first study using the NIH-TCB in a rural African setting. We reviewed the option of using several cognitive assessment tools including the Wechsler Abbreviated Scale of Intelligence (WASI-II) (135, 136), the Cambridge Neuropsychological Test Automated Battery (CANTAB test) (106, 137), and NIH-TCB (104, 105) in consultation with psychologists in our group. The decision was made to use the NIH-TCB since it was easier to use and less likely to be culturally sensitive. While acknowledging that cognitive tests originating from high socioeconomic contexts must be thoroughly adapted to local culture and language to ensure reliability and validity (136), not all tests may require adaptations for use across cultures. Processing speed and attention are, for instance, unlikely to be affected by unfamiliar content or language (138). The NIH-TCB automated trial tests also helped reduce any bias related to the speed of clicking by ensuring that the girls familiarized themselves with the actual test to be taken. Also, any bias related to the screen usage may have been reduced with the interviewers assisting girls who had problems with clicking on the screen during the trial test. Any clicking-related bias remaining in the cognition assessment would be evaluated in our statistical analyses by assessing interviewer as a possible confounder.

The randomized and follow-up design of our study allows us to examine associations and make causal inferences. Our use of different data-collection methods, including quantitative and qualitative methods, ensures data triangulation. Information obtained from the focus group discussions and in-depth interviews may help explain our psychosocial outcomes such as life satisfaction, aspirations, and quality of life. According to Drew et al. (139), the visual storytelling technique incorporated in the focus groups facilitates rich interviews, drawing out details of young peoples' lives that otherwise might not have been discussed. In the focus groups, the girls were asked to develop sketches of their thoughts about ideal body size and problematic body size, giving us a pictorial understanding of their perceptions.

Our recruitment and training of research assistants improved the quality of our data. Because the study did not include boys, most of the communities feared we were about to implement a family-planning program for their girls. Nevertheless, our prompt and regular engagement and sensitization of the community leaders, teachers, and the girls' mothers built community trust in the research team. More so, we ensured that a teacher who stayed in the community was trained and directly supervised the feeding of the girls, building more trust. The cluster supervisor and the team leader regularly visited and participated in feeding sessions to interact with the girls and address their fears and concerns in ensuring the trial's success. Our regular visits and participation in community programs made us a part of the communities in the study, further strengthening the trust. Additionally, our engagement of a medical practitioner who visited each community biweekly to examine and treat (when necessary) girls with any AEs also guaranteed more trust, ensuring that most of the girls were compliant.

We anticipated poor adherence to the RCT during the vacation periods, especially the long vacation period spanning mid-July to the end of August 2019. Most girls, particularly the older ones, often travel to southern Ghana for menial jobs during the period. Even so, the trust earned ensured that most of the parents encouraged their girls to stay and complete the study. That notwithstanding, most girls who were lost to follow-up traveled out of the area during this period.

Finally, implementing a 5-d run-in period allowed teachers and supervisors to practice the set-up, supervision, and completion of the daily case-report forms. The run-in feedback allowed the researchers to modify the case-report form to allow for ease of completion.

Trial status

The fieldwork has been completed, plasma samples have been analyzed, and we have begun data analysis. The descriptions largely reflect protocol version 2 submitted for ethical approval in May 2018. Recruitment into the RCT was between 18 February and 8 March 2019.

Declarations

Consent for publication and public disclosure.

The analysis and interpretation of the data and the decision to publish any articles from the data will be the authors' sole responsibility. According to established guidelines about authorship (International Committee of Medical Journal Editors), results will be reported in peer-reviewed international journals and reporting of RCTs. None of the funders had a role in study design and data-collection process.

Availability of data and materials.

Data collected are owned by Wageningen University and will be shared with the University for Development Studies (Ghana). Publications will include authors from all involved institutions based on their contribution. Data will be posted as open access on Data Archiving and Networked Services (DANS) 2 y after the study has been completed. Upon a reasonable request, data can be obtained from the leading author or Inge D. Brouwer (Inge.brouwer@wur.nl) from Wageningen UR.

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References

1. UNICEF. The state of the world's children 2019. Children, food and nutrition: growing well in a changing world. New York; UNICEF; 2019.
2. Ghana Statistical Service (GSS). 2010 Population and housing census report. Children, adolescents & young people in Ghana. Accra (Ghana): Ghana Statistical Service; 2013.
3. WHO. Health for the world's adolescents: a second chance in the second decade. Geneva (Switzerland): World Health Organization; 2014.
4. Prentice AM, Ward Ka, Goldberg GR, Jarjou LM, Moore SE, Fulford AJ, Prentice A. Critical windows for nutritional interventions against stunting. Am J Clin Nutr 2013;97:911–8.
5. Thurnham DI. Nutrition of adolescent girls in low- and middle-income countries. Sight Life 2013;27(3):26–37.
6. Soekarjo DD, Shulman S, Graciano F, Moench-Pfanner R. Improving nutrition for adolescent girls in Asia and the Middle East: innovations are needed. A joint collaboration between the Innovation Working Group Asia and One Goal. 2014 [Internet]. Available from: https://www.gainhealth.org/resources/reports-and-publications/improving-nutrition-adolescent-girls-asia-and-middle-east.
7. Spear BA. Adolescent growth and development. J Am Diet Assoc 2002;102:S23–9.
8. Stang J, Story M, editors. Guidelines for adolescent nutrition services. Minneapolis (MN): Center for Leadership, Education, and Training in Maternal and Child Nutrition Division of Epidemiology and Community Health School of Public Health, University of Minnesota; 2005.
9. Woodruff BA, Duffield A. Assessment of nutritional status in emergency-affected populations. United Nations Adm Comm Coord Sub-Committee Nutr. UN Syst Forum Nutr (Report from the The UN System's Forum for Nutrition) 2000;1–21.
10. WHO/FAO. Vitamin and mineral requirements in human nutrition. 2nd ed. Bangkok (Thailand): World Health Organization; 2004.
11. Arimond M, Wiesmann D, Becquey E, Carriquiry A, Daniels M, Deitchler M, Fanou N, Ferguson E, Joseph M, Kennedy G, et al. Dietary diversity as a measure of the micronutrient adequacy of women's diets in resource-poor areas: summary of results from five sites. Washington (DC): FHI 360; 2011.
12. Htet MK, Fahmida U, Dillon D, Akib A, Utomo B, Thurnham DI. The influence of vitamin A status on iron-deficiency anaemia in anaemic adolescent schoolgirls in Myanmar. Public Health Nutr 2013;17:1–8.
13. Hughes IA, Kumanan M. A wider perspective on puberty. Mol Cell Endocrinol 2006;254–255:1–7.
14. Martos-Moreno GA, Chowen JA, Argente J. Metabolic signals in human puberty: effects of over and undernutrition. Mol Cell Endocrinol 2010;324:70–81.
15. Zimmermann MB, Hurrell RF. Nutritional iron deficiency. Lancet North Am Ed 2007;370:511–20.
16. Zimmermann MB. Iodine deficiency. Endocr Rev 2009;30(4):376–408.
17. Mesías M, Seiquer I, Navarro MP. Iron nutrition in adolescence. Crit Rev Food Sci Nutr 2013;53:1226–37.
18. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. Am J Clin Nutr 2010;92:546–55.
19. Thurnham DI, Northrop-Clewes CA, Knowles J. The use of adjustment factors to address the impact of inflammation on vitamin A and iron status in humans. J Nutr 2015;145:1137S.
20. Karapanou O, Papadimitriou A. Determinants of menarche. Reprod Biol Endocrinol 2010;8:115.
21. Doss C. Intrahousehold bargaining and resource allocation in developing countries. 2013. The World Bank Policy Research Working Paper 6337.
22. Bingenheimer JB, Reed E. Risk for coerced sex among female youth in Ghana: roles of family context, school enrollment and relationship experience. IPSRH 2014;40:184–95.
23. FAO. Gender inequalities in rural employment in Ghana: an overview. 2012. The World Bank Policy Research Working Paper 6337.
24. Senadza B. Education inequality in Ghana: gender and spatial dimensions. J Econ Stud 2012;39:724–39.
25. UNICEF-Ghana. Out-of-school children. really simple stats: the UNICEF Ghana internal statistical bulletin. Out-of-School Children. 2015. The World Bank Policy Research Working Paper 6337.
26. Ghana Statistical Service (GSS)/Ghana Health Service (GHS)/ICF International. Demographic and Health Survey 2014. Ghana Statistical Service. Accra (Ghana) and Rockville (MD): Ghana Health Service and Ghana Statistical Service; 2015.

27. Korkalo L, Freese R, Alfthan G, Fidalgo L, Mutanen M. Poor micronutrient intake and status is a public health problem among adolescent Mozambican girls. Nutr Res 2015;35:664–73.
28. Lanerolle-Dias A, Lanerolle P, Arambepola C, Atukorala SM S, Lanerolle-Dias M, Silva A, Lanerolle P, Arambepola C, Atukorala S. Micronutrient status of female adolescent school dropouts. Ceylon Med J 2012;57:74–8.
29. Black RE, Allen LH, Bhutta ZA, Caulfield LE, de Onis M, Ezzati M, Mathers C, Rivera J. Maternal and child undernutrition: global and regional exposures and health consequences. Lancet North Am Ed 2008;371: 243–60.
30. Azupogo F, Aurino E, Gelli A, Bosompem KM, Ayi I, Osendarp SJM, Brouwer ID, Folson G. Agro-ecological zone and farm diversity are factors associated with haemoglobin and anaemia among rural school-aged children and adolescents in Ghana. Matern Child Nutr 2018;15(1):e12643.
31. Keats EC, Rappaport AI, Shah S, Oh C, Jain R, Bhutta ZA. The dietary intake and practices of adolescent girls in low-and middle-income countries: a systematic review. Nutrients 2018;10;1978.
32. Torheim LE, Ferguson EL, Penrose K, Arimond M. Women in resource-poor settings are at risk of inadequate intakes of multiple micronutrients. J Nutr 2010;140:2051S–8S.
33. WHO. Adolescent nutrition: a review of the situation in selected South-East Asian countries. New Delhi (India): World Health Organization; 2006.
34. Anonymous. Adolescence: a second chance to tackle inequities. Lancet North Am Ed;2013;382:1535.
35. World Health Organization. Guideline: intermittent iron and folic acid supplementation in menstruating women. Geneva (Switzerland): World Health Organization; 2011.
36. Semba RD, Bloem MW. Nutrition and health in developing countries. 2nd ed. Totowa (NJ): Humana Press; 2008.
37. WHO/FAO. Guidelines on food fortification with micronutrients. Allen L, Benoist B, Dary O, Hurrell R, editors. Geneva (Switzerland): World Health Organization and Food and Agriculture Organization of the United Nations; 2006.
38. Berner LA, Keast DR, Bailey RL, Dwyer JT. Fortified foods are major contributors to nutrient intakes in diets of US children and adolescents. J Acad Nutr Diet 2014;114:1009–22, e8.
39. Kersting M, Alexy U, Manz F. Ten-year trends in vitamin and mineral intake from fortified food in German children and adolescents. Eur J Clin Nutr 2000;54:81–6.
40. Powers HJ, Stephens M, Russell J, Hill MH. Fortified breakfast cereal consumed daily for 12 wk leads to a significant improvement in micronutrient intake and micronutrient status in adolescent girls: a randomised controlled trial. Nutr J 2015;15:69.
41. Wang X, Hui Z, Dai X, Terry PD, Zhang Y, Ma M, Wang M, Deng F, Gu W, Lei S, et al. Micronutrient-fortified milk and academic performance among Chinese middle school students: a cluster-randomized controlled trial. Nutrients 2017;9:226.
42. Madsen KH, Rasmussen LB, Andersen R, Mølgaard C, Jakobsen J, Bjerrum PJ, Andersen EW, Mejborn H, Tetens I. Randomized controlled trial of the effects of vitamin D—fortified milk and bread on serum 25-hydroxyvitamin D concentrations in families in Denmark during winter : the VitmaD study. Am J Clin Nutr 2013;98:374–82.
43. Hyder SMZ, Haseen F, Khan M, Schaetzel T, Jalal CSB, Rahman M, Lo B, Mannar V, Mehansho H. A multiple-micronutrient-fortified beverage affects hemoglobin, iron, and vitamin A status and growth in adolescent girls in rural Bangladesh. J Nutr 2007;137:2147–53.
44. Alayne MA, Rushdia A, Mahbub Latif AHM, Sabrina R, Sumon K D, Enamul H, Fahmida DF, Farzana F, Shahnawaz A, Asg F. Impact of fortified biscuits on micronutrient deficiencies among primary school children in Bangladesh. PLoS One 2017;12:1–16.
45. Goyle A, Prakash S. Effect of supplementation of micronutrient fortified biscuits on serum total proteins and vitamin A levels of adolescent girls (10–16 years) of Jaipur city, India. Nepal Med Coll J 2011;13:233–7.
46. Blum LS, Mellisa A, Kurnia Sari E, Novitasari Yusadiredja I, van Liere M, Shulman S, Izwardy D, Menon R, Tumilowicz A. In-depth assessment of snacking behaviour in unmarried adolescent girls 16–19 years of age living in urban centres of Java, Indonesia. Matern Child Nutr 2019;15:1–12.
47. Perkins JM, Subramanian SV, Smith GD, Özaltin E. Adult height, nutrition, and population health. Nutr Rev 2016;74:149–65.
48. Schaible UE, Kaufmann SHE. Malnutrition and infection: complex mechanisms and global impacts. PLoS Med 2007;4:e115.
49. Robinson SL, Marín C, Oliveros H, Mora-Plazas M, Richards BJ, Lozoff B, Villamor E. Iron deficiency, anemia, and low vitamin B-12 serostatus in middle childhood are associated with behavior problems in adolescent boys: results from the Bogotá School Children Cohort. J Nutr 2018;148: 760–70.
50. Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. Glia 2009;57:467–78.
51. Beard J. Iron deficiency alters brain development and functioning. J Nutr 2003;133(S5):1468S–72S.
52. Zimmermann MB, Connolly K, Bozo M, Bridson J, Rohner F, Grimci L. Iodine supplementation improves cognition in iodine-deficient schoolchildren in Albania: a randomized, controlled, double-blind study. Am J Clin Nutr 2006;83:108–14.
53. Zimmermann MB, Jooste PL, Mabapa NS, Schoeman S, Biebinger R, Mushaphi LF, Mbhenyane X. Vitamin A supplementation in iodine-deficient African children decreases thyrotropin stimulation of the thyroid and reduces the goiter rate. Am J Clin Nutr 2007.861040
54. Beard JL, Wiesinger JA, Connor JR. Pre- and postweaning iron deficiency alters myelination in Sprague-Dawley rats. Dev Neurosci 2003;25: 308–15.
55. Dercon S, Sánchez A. Height in mid childhood and psychosocial competencies in late childhood: evidence from four developing countries. Econ Hum Biol 2013;11:426–32.
56. Casas AG, Guillamon AR, Garcia-Cantò E, Rodrìguez Garcìa PL, Pèrez-Soto JJ, Marcos LT, Lòpez PT. Estado nutricional y calidad de vida relacionada con la salud en escolares del sureste español. Nutr Hosp 2015;31:737–43.
57. Helseth S, Haraldstad K, Christophersen KA. A cross-sectional study of health related quality of life and body mass index in a Norwegian school sample (8–18 years): a comparison of child and parent perspectives. Health Qual Life Outcomes 2015;13:47.
58. Crookston R, McClellan C, Georgiadis A, Heaton T. Factors associated with cognitive achievement in late childhood and adolescence: the Young Lives cohort study of children in Ethiopia, India, Peru, and Vietnam. BMC Pediatr 2014;14.
59. Dissanayake DS, Kumarasiri PVR, Nugegoda DB, Dissanayake DM. The association of iron status with educational performance and intelligence among adolescents. Ceylon Med J 2009;54:75.
60. Fink G, Rockers PC. Childhood growth, schooling, and cognitive development: further evidence from the Young Lives Study. Am J Clin Nutr 2014;100(1):182–8.
61. Teni M, Shiferaw S, Asefa F. Anemia and its relationship with academic performance among adolescent school girls in Kebena District, Southwest Ethiopia. Biotech Health Sci 2017;4(1).
62. Acham H, Kikafunda JK, Oluka S, Malde MK, Tylleskar T. Height, weight, body mass index and learning achievement in Kumi district, east of Uganda. Sci Res Essays 2008;3:1–8.
63. Riley AP. Determinants of adolescent fertility and its consequences for maternal health, with special reference to rural Bangladesh. Ann N Y Acad Sci 1994;709:86–100.
64. Christian P, Smith ER. Adolescent undernutrition: global burden, physiology, and nutritional risks. Ann Nutr Metab 2018;72:316–28.
65. UNICEF. The Global Strategy for Women's, Children's and Adolescents' Health (2016–2030). Geneva (Switzerland): UNICEF; 2016.
66. Hardgrove A, Pells K, Boyden J, Dornan P. Youth vulnerabilities in life course transitions. 2014 UNDP Human Development Report Office Occasional paper. New York: United Nations Development Programme; 2014.
67. Mensch BS, Bruce J, Greene ME. The uncharted passage: girls' adolescence in the developing world. New York: The Population Council, Inc.; 1998.
68. Dercon S, Singh A. From nutrition to aspirations and self-efficacy: gender bias over time among children in four countries. World Dev 2013;45: 31–50.

69. Saaka M. Women's decision-making autonomy and its relationship with child feeding practices and postnatal growth. J Nutr Sci 2020;9: 1–10.
70. Black RE, Victora CG, Walker SP, Bhutta Za, Christian P, De Onis M, Ezzati M, Grantham-Mcgregor S, Katz J, Martorell R, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet North Am Ed 2013;382:427–51.
71. Rahman MM, Saima U, Goni MA. Impact of maternal household decision-making autonomy on child nutritional status in Bangladesh. Asia Pac J Public Health 2015;27:509–20.
72. Ghana Statistical Service (GSS). 2010 Population and Housing Census. District analytical report. Mion District. Accra(Ghana): Ghana Statistical Service; 2014.
73. Angelse-Agdeppa, Schulthink W, Sastroamidjojo S, Gross R, Karyadi D. Weekly micronutrient supplementation to build iron in female Indonesian adolescents. Am J Clin Nutr 1997;66:177–83.
74. Mwaniki D, Omondi B, Muniu E, Thiong'o F, Ouma J, Magnussen P, Geissler PW, Michaelsen KF, Friis H. Effects on haemoglobin of multi-micronutrient supplementation and multi-helminth chemotherapy: a randomized, controlled trial in Kenyan school children. Eur J Clin Nutr 2003;57:573–9.
75. Bhaumik DK, Roy A, Aryal S, Hur K, Duan N, Normand S-LT, Brown CH, Gibbons RD. Sample size determination for studies with repeated continuous outcomes. Psychiatr Ann 2008;38:765–71.
76. Patimah S, As S, Hadju V, Thaha AR. The efficacy of multiple micronutrient supplementation on improvement hemoglobin and serum feritin level in adolescent girls with anemia. Int J Sci Res Publ 2014;4:1–8.
77. Danquah AO, Amoah AN, Opare-Obisaw C. Nutritional status of upper primary school pupils in a rural setting in Ghana. IJNFS 2013;2:320.
78. Rothman KJ. Epidemiology: an introduction. 2nd ed. New York: Oxford University Press; 2012.
79. WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Geneva (Switzerland): WHO; 2011.
80. Bernard RH. Research methods in anthropology: qualitative and quantitative approaches. 4th ed. New York: Altamira Press; 2006.
81. Wyatt TH, Krauskopf PB, Davidson R. Using focus groups for program planning and evaluation. J Sch Nurs 2008;24:71–82.
82. UNICEF. UNICEF annual report 2017 Ghana. Accra (Ghana): UNICEF; 2017.
83. Ameade EPK, Garti HA. Age at menarche and factors that influence it: a study among female university students in Tamale, Northern Ghana. PLoS One 2016;11:e0155310.
84. Gumanga SK, Kwame-Aryee RA. Menstrual characteristics in some adolescent girls in Accra, Ghana. Ghana Med J 2012;46:3–7.
85. Richmond A, Anthony A, Martin A. Age of menarche among basic level school girls in Madina, Accra. Afr J Reprod Health 2011;15:110–3.
86. Ghana Ministry of Health. Guidelines for case management of malaria in Ghana. Accra (Ghana): Ghana Health Service; 2014.
87. van Stuijvenberg M, Dhansay M, Smuts C, Lombard C, Jogessar V, Benadé A. Long-term evaluation of a micronutrient-fortified biscuit used for addressing micronutrient deficiencies in primary school children. Public Health Nutr 2001;4:1201–9.
88. Erhardt JG, Estes JE, Pfeiffer CM, Biesalski HK, Craft NE. Combined measurement of ferritin, soluble transferrin receptor, retinol binding protein, and C-reactive protein by an inexpensive, sensitive, and simple sandwich enzyme-linked immunosorbent assay technique. J Nutr 2004;134:3127–32.
89. Gibson RS, Ferguson EL. An interactive 24-hour recall for assessing the adequacy of iron and zinc intakes in developing countries.HarvestPlus Technical Monographs. Washington (DC): ILSI Press; 2008.
90. Cashin K, Oot L. Guide to anthropometry: a practical tool for program planners, managers, and implementers. Washington (DC): Food and Nutrition Technical Assistance III Project (FANTA)/FHI 360; 2018.
91. Akern. Bodygram PLUS software v. 1.2.2.8. Lisbon (Portugal): Akern; 2014.
92. FAO; FANTA III. Minimum dietary diversity for women: a guide to measurement. Food and Nutrition Technical Assistance III. Rome (Italy): FAO and FHI 360; 2016.
93. FAO. The Food Insecurity Experience Scale (FIES). Guidance for translation: intended meanings of the questions and specific terms. Rome (Italy): FAO; 2015.
94. Crockett LJ, Petersen AC. Pubertal status and psychosocial development: findings from the Early Adolescence Study. Biol Interact Early Adolesc Interact Early Adolesc 1987;227:173–88.
95. Carskadon MA, Acebo C. A self-administered rating scale for pubertal development. J Adolesc Health 1993;14:190–5.
96. Ravens-Sieberer U, Gosch A, Rajmil L, Erhart M, Bruil J, Power M, Duer W, Auquier P, Cloetta B, Czemy L, et al. The KIDSCREEN-52 quality of life measure for children and adolescents: psychometric results from a cross-cultural survey in 13 European countries. Qual Life Res 2008;11: 645–58.
97. Ravens-Sieberer U, Herdman M, Devine J, Otto C, Bullinger M, Rose M, Klasen F. The European KIDSCREEN approach to measure quality of life and well-being in children: development, current application, and future advances. Qual Life Res 2014;23:791–803.
98. Currie C, Roberts C, Morgan A, Smith R, Settertobulte W, Samdal O, editors. Young people's health in context. Health Behavior in School-aged Children (HBSC) Study: international report from the 2001/2002 survey. Health Policy for Children and Adolescents. Copenhagen (Denmark): WHO; 2002.
99. Rosenberg M. Society and the adolescent self-image. Princeton (NJ): University Press; 1965.
100. Chen G, Gully, Stanley M, Eden D. Validation of a new general self-efficacy scale. Organizational Research Methods 2001;4:62–83.
101. OECD. Society at a glance 2019: OECD social indicators. Paris: OECD Publishing; 2019.
102. Stunkard AJ, Sørensen T, Schulsinger F. Use of the Danish Adoption Register for the study of obesity and thinness. Res Publ Assoc Res Nerv Ment Dis 1983;60:115–20.
103. Vega R , Racine M, Sánchez-Rodríguez E, Solé E, Castarlenas E, Jensen MP, Engel J, Miró J. Psychometric properties of the short form of the Children's Depression Inventory (CDI-S) in young people with physical disabilities. J Psychosom Res 2016;90:57–61.
104. Zelazo PD, Anderson JE, Richler J, Wallner-Allen K, Beaumont JL, Weintraub S. NIH Toolbox Cognition Battery (Cb): measuring executive function and attention. Monographs Society Res Child 2013;78: 16–33.
105. Gershon RC, Wagster MV, Hendrie HC, Fox NA, Cook KF, Nowinski CJ. NIH Toolbox for assessment of neurological and behavioral function. Neurology 2013;80:S2–6.
106. Gau SSF, Shang CY. Executive functions as endophenotypes in ADHD: wvidence from the Cambridge Neuropsychological Test Battery (CANTAB). J Child Psychol Psychiatry 2010;51:838–49.
107. Ismatullina V, Voronin I, Shelemetieva A, Malykh S. Cross-cultural Study of Working Memory in Adolescents. Procedia Soc Behav Sci 2014;146: 353–7.
108. Barnett I, Ariana P, Petrou S, Penny ME, Duc LT, Galab S, Woldehanna T, Escoba JA, Plugge E, Boyden J, et al. Cohort profile: the Young Lives Study. Int J Epidemiol 2013;42:701–8.
109. Harris-Fry H, Paudel P, Karn M, Mishra N, Thakur J, Paudel V, Harrisson T, Shrestha B, Manandhar DS, Costello A, et al. Development and validation of a photographic food atlas for portion size assessment in the southern plains of Nepal. Public Health Nutr 2016;19:2495–507.
110. Demographic and Health Surveys. Guide to DHS statistics. Demographic and Health Surveys Methodology. Rutstein SO, Rojas G, editors. Calverton (MD): Demographic and Health Surveys, ORC Macro; 2006.
111. USAID. Standard recode manual for DHS 6. Demographic and Health Survey Methodology. Calverton (MD): ORC Macro; 2013.
112. Mack N, Woodsong C, McQueen KM, Guest G, Namey E. Qualitative research methods: a data collector's field guide. Durham (NC): Family Health International (FHI); 2005.
113. Boyce C, Neale P. Conducting in-depth interviews: a guide for designing and conducting in-depth interviews for evaluation input. Pathfinder International Tool Series: Monitoring and Evaluation–2. Watertown (MA): Pathfinder International; 2006.

114. Field A, Miles J. Discovering statistics using SAS. Los Angeles (CA): Sage Publications; 2010.
115. West BT, Welch KB, Galecki AT. Linear mixed models: a practical guide using statistical software. Boca Raton (FL): Taylor & Francis; 2007.
116. Barros AJD, Hirakata VN. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. BMC Med Res Methodol 2003;3:1–13.
117. Coutinho LMS, Scazufca M, Menezes PR. Methods for estimating prevalence ratios in cross-sectional studies. Rev Saude Publica 2008;42: 992–8.
118. Webb P, Bain C. Essential epidemiology: an introduction for students and health professionals. 2nd ed. Cambridge (UK): Cambridge University Press; 2010.
119. Berglund P, Heeringa S. Multiple imputation of missing data using SAS. Cary (NC): SAS Institute; 2014.
120. Dekkers AL, Verkaik-Kloosterman J, van Rossum CT, Ocké MC. SPADE, a new statistical program to estimate habitual dietary intake from multiple food sources and dietary supplements. J Nutr 2014;144: 2083–91.
121. Allen LH, Carriquiry AL, Murphy SP. Perspective: proposed harmonized nutrient reference values for populations. Adv Nutr 2020;11:469–83.
122. Crampton K. Optifood user manual. Version 0.4. Geneva (Switzerland): WHO and FANTA II; 2011.
123. Ferguson EL, Darmon N, Premachandra IM, Al FET. Food-based dietary guidelines can be developed and tested using linear programming analysis. J Nutr 2004;134:951–7.
124. Aurino E. Do boys eat better than girls in India? Longitudinal evidence on dietary diversity and food consumption disparities among children and adolescents. Econ Hum Biol 2017;25:99–111.
125. Hayes N. Doing psychological research: gathering and analyzing data. In: Doing qualitative analysis in psychology. Philadelphia (PA): Open University Press; 2000. pp. 93–114.
126. Braun V, Clarke V. Using thematic analysis in psychology. Qual Res Psychol 2006;3:77–101.
127. Das JK, Salam RA, Kumar R, Bhutta ZA. Micronutrient fortification of food and its impact on woman and child health: a systematic review. Syst Rev 2013;2:67.
128. Koury MJ, Ponka P. New insights into erythropoiesis: the roles of folate, vitamin B12, and iron. Annu Rev Nutr 2004;24:105–31.
129. Talsma EF, Verhoef H, Brouwer ID, Wagt ASM, Hulshof PJM, Melse-Boonstra A, Mburu-de Wagt AS, Hulshof PJM, Melse-Boonstra A. Proxy markers of serum retinol concentration, used alone and in combination, to assess population vitamin A status in Kenyan children: a cross-sectional study. BMC Med 2015;13:30.
130. Larson LM, Namaste SM, Williams AM, Engle-Stone R, Addo OY, Suchdev PS, Wirth JP, Temple V, Serdula M, Northrop-Clewes CA. Adjusting retinol-binding protein concentrations for inflammation: Biomarkers Reflecting Inflammation and Nutritional Determinants of Anemia (BRINDA) project. Am J Clin Nutr 2017;106:390S–401S.
131. Stoltzfus RJ, Dreyfuss ML. Guidelines for the use of iron supplements to prevent and treat iron deficiency anemia. Pediatrics. Washington (DC): World Health Organization/UNICEF/International Nutritional Anemia Consultative Group; 1998.
132. Ahmed F, Khan MR, Akhtaruzzaman M, Karim R, Williams G, Banu CP, Nahar B, Darnton-Hill I. Effect of long-term intermittent supplementation with multiple micronutrients compared with iron-and-folic acid supplementation on Hb and micronutrient status of non-anaemic adolescent schoolgirls in rural Bangladesh. Br J Nutr 2012;108:1484–93.
133. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomized study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet North Am Ed 1996;348:992–6.
134. Whitehead-Jr RD, Mei Z, Mapango C, Jefferds MED. Methods and analyzers for hemoglobin measurement in clinical laboratories and field settings. Ann N Y Acad Sci 2019;1450:147–71.
135. McCrimmon AW, Smith AD. Test review of the Wechsler Abbreviated Scale of Intelligence, Second Edition. J Psychoeducation Assess 2013;31: 337–41.
136. Isaacs E, Oates J. Nutrition and cognition: assessing cognitive abilities in children and young people. Eur J Nutr 2008;47:4–24.
137. Telléus GK, Jepsen JR, Bentz M, Christiansen E, Jensen SOW, Fagerlund B, Thomsen PH. Cognitive profile of children and adolescents with anorexia nervosa. Eur Eat Disorders Rev 2015;23:34–42.
138. Hughes D, Bryan J. The assessment of cognitive performance in children: considerations for detecting nutritional influences. Nutr Rev 2003;61: 413–22.
139. Drew SE, Duncan RE, Sawyer SM. Visual storytelling: a beneficial but challenging method for health research with young people. Qual Health Res 2010;20:1677–88.